PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 25 January 2001 (25.01.01)	
International application No.: PCT/JP00/04683	Applicant's or agent's file reference: 2638WO0P
International filing date: 13 July 2000 (13.07.00)	Priority date: 15 July 1999 (15.07.99)
Applicant: IGARI, Yasutaka et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

14 September 2000 (14.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2638WO0P	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP00/04683	International filing date (*day/month/year*) 13 July 2000 (13.07.00)	Priority date (*day/month/year*) 15 July 1999 (15.07.99)
International Patent Classification (IPC) or national classification and IPC A61K 9/52, 38/09, 47/12, 47/34, A61P 35/00, 5/24, 13/08, 15/00		
Applicant TAKEDA CHEMICAL INDUSTRIES, LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 14 September 2000 (14.09.00)	Date of completion of this report 27 June 2001 (27.06.2001)
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP00/04683

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP 00/04683

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-23	YES
	Claims		NO
Inventive step (IS)	Claims	1-23	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-23	YES
	Claims		NO

2. Citations and explanations

Document 1: WO, 98/32423, A1 (Takeda Chemical Industries, Ltd.), 30 July 1998

Document 2: WO, 96/22786, A1 (Takeda Chemical Industries, Ltd.), 1 August 1996

Claims 1-15 and 22

The inventions described in Claims 1-15 and 22 are novel and involve an inventive step relative to Documents 1 and 2 cited in the international search report.

Neither Document 1 nor Document 2 discloses sustained release compositions containing (1) a physiologically active substance or a salt thereof, (2) hydroxynaphthoic acid or a salt thereof, and (3) a lactic acid/glycolic acid polymer or salt thereof in which value for the weight average molecular weight multiplied by the quantity of carboxyl end groups per unit mass is within a certain range, or a salt thereof (the aforementioned three ingredients are referred to hereafter as the "three specified ingredients"). Moreover, by including the aforementioned three specified ingredients, the inventions described in these claims offer the advantageous effect that excess initial release of the physiologically active substance is controlled, giving persistent release over an unusually long time.

Claims 16-19

The inventions described in Claims 16-19 are novel and involve an inventive step relative to Documents 1 and 2 cited in the international search report.

Neither Document 1 nor Document 2 cited in the international search report discloses a process for producing a gradual release composition by removing the solvent from a mixed solution of the aforementioned three specified ingredients, nor could this be deduced easily by a person skilled in the art, since the gradual release substances containing said three specified ingredients which are the object of the production process are not known.

Claims 20 and 21

The inventions described in Claims 20 and 21 are novel and involve an inventive step relative to Documents 1 and 2 cited in the international search report.

Neither Document 1 nor Document 2 discloses a pharmaceutical including a gradual release composition containing the aforementioned three specified ingredients, nor could this be deduced easily by a person skilled in the art, since gradual release substances containing said three specified ingredients enveloping a pharmacologically active ingredient are not known.

Claim 23

The invention described in Claim 23 is novel and involves an inventive step relative to Documents 1 and 2 cited in the international search report.

Neither Document 1 nor Document 2 discloses a gradual release composition containing a physiologically active substance or a salt thereof, the specific hydroxy-naphthoic acid 1-hydroxy-2-naphthoic acid or a salt

thereof, and a biodegradable polymer or a salt thereof. Moreover, the choice of said specific hydroxynaphthoic acid as an ingredient of a gradual release composition is not obvious within the art.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO,99/36099,A1 [E,X]	22 July 1999 (22.07.1999)	13 January 1999 (13.01.1999)	16 January 1998 (16.01.1998)

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Claim 23 is not fully supported by the description.

Observation

The only biodegradable polymers described in the description of the present application are lactic acid/-glycolic acid polymers in which value for the weight average molecular weight multiplied by the quantity of carboxyl end groups per unit mass is within a certain range; no mention is made of other biodegradable polymers, and no examples thereof are given.

The account in the description indicates that the gradual release of the physiologically substance is closely associated with the physical and chemical properties of the aforementioned three specified ingredients, and it is not obvious from knowledge of the art at the time of filing the present application that use of any other biodegradable polymer will offer the same effects as the aforementioned specified polymers.

14T

特 許 協 力 条 約

PCT

国際予備審査報告

(法第１２条、法施行規則第56条)
〔ＰＣＴ３６条及びＰＣＴ規則70〕

出願人又は代理人の書類記号　２６３８ＷＯＯＰ	今後の手続きについては、国際予備審査報告の送付通知（様式ＰＣＴ／ＩＰＥＡ／４１６）を参照すること。	
国際出願番号 ＰＣＴ／ＪＰ００／０４６８３	国際出願日 (日.月.年)　１３．０７．００	優先日 (日.月.年)　１５．０７．９９
国際特許分類（ＩＰＣ）　Ｉｎｔ．　Ｃｌ' Ａ６１Ｋ９／５２，Ａ６１Ｋ３８／０９，Ａ６１Ｋ４７／１２，Ａ６１Ｋ４７／３４，Ａ６１Ｐ３５／００，Ａ６１Ｐ５／２４，Ａ６１Ｐ１３／０８，Ａ６１Ｐ１５／００		
出願人（氏名又は名称）　武田薬品工業株式会社		

1．　国際予備審査機関が作成したこの国際予備審査報告を法施行規則第57条（ＰＣＴ３６条）の規定に従い送付する。

2．　この国際予備審査報告は、この表紙を含めて全部で　6　ページからなる。

☐ この国際予備審査報告には、附属書類、つまり補正されて、この報告の基礎とされた及び／又はこの国際予備審査機関に対してした訂正を含む明細書、請求の範囲及び／又は図面も添付されている。
（ＰＣＴ規則70.16及びＰＣＴ実施細則第６０７号参照）
この附属書類は、全部で＿＿＿＿＿＿ページである。

3．　この国際予備審査報告は、次の内容を含む。

I ☒ 国際予備審査報告の基礎

II ☐ 優先権

III ☐ 新規性、進歩性又は産業上の利用可能性についての国際予備審査報告の不作成

IV ☐ 発明の単一性の欠如

V ☒ ＰＣＴ３５条(2)に規定する新規性、進歩性又は産業上の利用可能性についての見解、それを裏付けるための文献及び説明

VI ☒ ある種の引用文献

VII ☐ 国際出願の不備

VIII ☒ 国際出願に対する意見

国際予備審査の請求書を受理した日　１４．０９．００	国際予備審査報告を作成した日　２７．０６．０１	
名称及びあて先 日本国特許庁（ＩＰＥＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員） 高原　慎太郎	４Ｃ　３０３９
	電話番号　０３－３５８１－１１０１　内線　３４５２	

様式ＰＣＴ／ＩＰＥＡ／４０９（表紙）（１９９８年７月）

国際予備審査報告

国際出願番号 PCT／JP00／04683

Ⅰ. 国際予備審査報告の基礎

1. この国際予備審査報告は下記の出願書類に基づいて作成された。（法第６条（ＰＣＴ１４条）の規定に基づく命令に応答するために提出された差し替え用紙は、この報告書において「出願時」とし、本報告書には添付しない。
ＰＣＴ規則70.16, 70.17）

☒ 出願時の国際出願書類

☐ 明細書 第 ＿＿＿＿＿＿ ページ、 出願時に提出されたもの
明細書 第 ＿＿＿＿＿＿ ページ、 国際予備審査の請求書と共に提出されたもの
明細書 第 ＿＿＿＿＿＿ ページ、 ＿＿＿＿＿＿ 付の書簡と共に提出されたもの

☐ 請求の範囲 第 ＿＿＿＿＿＿ 項、 出願時に提出されたもの
請求の範囲 第 ＿＿＿＿＿＿ 項、 ＰＣＴ１９条の規定に基づき補正されたもの
請求の範囲 第 ＿＿＿＿＿＿ 項、 国際予備審査の請求書と共に提出されたもの
請求の範囲 第 ＿＿＿＿＿＿ 項、 ＿＿＿＿＿＿ 付の書簡と共に提出されたもの

☐ 図面 第 ＿＿＿＿＿＿ ページ／図、 出願時に提出されたもの
図面 第 ＿＿＿＿＿＿ ページ／図、 国際予備審査の請求書と共に提出されたもの
図面 第 ＿＿＿＿＿＿ ページ／図、 ＿＿＿＿＿＿ 付の書簡と共に提出されたもの

☐ 明細書の配列表の部分 第＿＿＿＿＿＿ ページ、 出願時に提出されたもの
明細書の配列表の部分 第＿＿＿＿＿＿ ページ、 国際予備審査の請求書と共に提出されたもの
明細書の配列表の部分 第＿＿＿＿＿＿ ページ、 ＿＿＿＿＿＿ 付の書簡と共に提出されたもの

2. 上記の出願書類の言語は、下記に示す場合を除くほか、この国際出願の言語である。

上記の書類は、下記の言語である ＿＿＿＿＿＿ 語である。

☐ 国際調査のために提出されたＰＣＴ規則23.1(b)にいう翻訳文の言語
☐ ＰＣＴ規則48.3(b)にいう国際公開の言語
☐ 国際予備審査のために提出されたＰＣＴ規則55.2または55.3にいう翻訳文の言語

3. この国際出願は、ヌクレオチド又はアミノ酸配列を含んでおり、次の配列表に基づき国際予備審査報告を行った。

☐ この国際出願に含まれる書面による配列表
☐ この国際出願と共に提出されたフレキシブルディスクによる配列表
☐ 出願後に、この国際予備審査（または調査）機関に提出された書面による配列表
☐ 出願後に、この国際予備審査（または調査）機関に提出されたフレキシブルディスクによる配列表
☐ 出願後に提出した書面による配列表が出願時における国際出願の開示の範囲を超える事項を含まない旨の陳述書の提出があった
☐ 書面による配列表に記載した配列とフレキシブルディスクによる配列表に記録した配列が同一である旨の陳述書の提出があった。

4. 補正により、下記の書類が削除された。
☐ 明細書 第 ＿＿＿＿＿＿ ページ
☐ 請求の範囲 第 ＿＿＿＿＿＿ 項
☐ 図面 図面の第 ＿＿＿＿＿＿ ページ／図

5. ☐ この国際予備審査報告は、補充欄に示したように、補正が出願時における開示の範囲を越えてされたものと認められるので、その補正がされなかったものとして作成した。（ＰＣＴ規則70.2(c) この補正を含む差し替え用紙は上記１．における判断の際に考慮しなければならず、本報告に添付する。）

国際予備審査報告

国際出願番号 PCT／JP00／04683

V. 新規性、進歩性又は産業上の利用可能性についての法第１２条（ＰＣＴ３５条(2)）に定める見解、それを裏付ける文献及び説明

1. 見解

新規性（N）	請求の範囲	1－23	有
	請求の範囲		無
進歩性（ＩＳ）	請求の範囲	1－23	有
	請求の範囲		無
産業上の利用可能性（ＩＡ）	請求の範囲	1－23	有
	請求の範囲		無

2. 文献及び説明（ＰＣＴ規則70.7）

文献１： WO,98/32423, A1(TAKEDA CHEMICAL INDUSTRIES,LTD.) 30.7月.1998
文献２： WO,96/22786, A1(TAKEDA CHEMICAL INDUSTRIES,LTD.) 1.8月.1996

請求の範囲１－１５，２２
請求の範囲１－１５，２２に係る発明は、国際調査報告で引用された文献１，２に対して新規性、進歩性を有する。
生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩、及び重量平均分子量と単位質量当たりの末端カルボキシル基量との積が特定範囲である乳酸－グリコール酸重合体またはその塩（以下、上記３成分を「特定３成分」という。）を含有する徐放性組成物は文献１，２には開示されていない。また、本請求項に係る発明は、上記特定３成分を併用することにより、生理活性物質の初期過剰放出を抑制して、非常に長期にわたる持続放出を実現させるという有利な効果を発揮するものである。

請求の範囲１６－１９
請求の範囲１６－１９に係る発明は、国際調査報告で引用された文献１，２に対して新規性、進歩性を有する。
上記特定３成分の混合液から溶媒を除去することにより徐放性組成物を製造する方法は、国際調査報告で引用された文献１，２には開示されておらず、また、製造目的物である当該特定３成分を含有する徐放性物質が公知のものとも認められないので、当業者といえども容易に想到し得ないものである。

請求の範囲２０，２１
請求の範囲２０，２１に係る発明は、国際調査報告で引用された文献１，２に対して新規性、進歩性を有する。
上記特定３成分を含有する徐放性組成物を配合した医薬は、国際調査報告で引用された文献１，２には開示されておらず、また、薬理活性成分を包含している当該特定３成分を含有する徐放性物質が公知のものとも認められないので、当業者といえども容易に想到し得ないものである。

VI.　ある種の引用文献

1.　ある種の公表された文書（PCT規則70.10）

出願番号 特許番号	公知日 （日．月．年）	出願日 （日．月．年）	優先日（有効な優先権の主張） （日．月．年）
WO,99/36099,A1 「E，X」	22.07.99	13.01.99	16.01.98

2.　書面による開示以外の開示（PCT規則70.9）

書面による開示以外の開示の種類	書面による開示以外の開示の日付 （日．月．年）	書面による開示以外の開示に言及している書面の日付（日．月．年）

Ⅷ.　国際出願に対する意見

請求の範囲、明細書及び図面の明瞭性又は請求の範囲の明細書による十分な裏付についての意見を次に示す。

1．請求の範囲２３に関して、明細書による十分な裏付がされていない。

備考：
本願明細書中では、生体内分解性ポリマーとして、重量平均分子量と単位質量当たりの末端カルボキシル基量との積が特定範囲である乳酸－グリコール酸重合体（以下、「特定ポリマー」という。）が記載されているのみであり、その他の生分解性ポリマーについては例示も含め、何ら触れるところがない。
本願明細書の記載をみるに、生理活性物質の徐放作用には上記特定ポリマーの有する物理・化学的性質が密接に関与していると認められるが、本願出願時の技術常識を参酌しても、その他の任意の生体内分解性ポリマーを用いた場合にも、上記特定ポリマーと同等の効果を奏することが、自明であるとは認められない。

補充欄（いずれかの欄の大きさが足りない場合に使用すること）

第　V　欄の続き

請求の範囲２３
　請求の範囲２３に係る発明は、国際調査報告で引用された文献１，２に対して新規性、進歩性を有する。
　生理活性物質またはその塩、１－ヒドロキシー２－ナフトエ酸という特定のヒドロキシナフトエ酸またはその塩、および生体内分解性ポリマーまたはその塩を含有する徐放性組成物は、国際調査報告で引用された文献１，２には開示されていない。また、徐放性組成物の配合成分として当該特定のヒドロキシナフトエ酸を選択することが自明の技術的事項であるとも認められない。

15

EP ・ US

特 許 協 力 条 約

PCT

国際調査報告

（法８条、法施行規則第40、41条）
〔ＰＣＴ１８条、ＰＣＴ規則43、44〕

出願人又は代理人 の書類記号　２６３８ＷＯＯＰ	今後の手続きについては、国際調査報告の送付通知様式（ＰＣＴ／ＩＳＡ／２２０）及び下記５を参照すること。	
国際出願番号 ＰＣＴ／ＪＰ００／０４６８３	国際出願日 （日.月.年）　１３．０７．００	優先日 （日.月.年）　１５．０７．９９
出願人（氏名又は名称） 武田薬品工業株式会社		

国際調査機関が作成したこの国際調査報告を法施行規則第41条（ＰＣＴ１８条）の規定に従い出願人に送付する。
この写しは国際事務局にも送付される。

この国際調査報告は、全部で　3　ページである。

☐ この調査報告に引用された先行技術文献の写しも添付されている。

１．国際調査報告の基礎

ａ．言語は、下記に示す場合を除くほか、この国際出願がされたものに基づき国際調査を行った。
☐ この国際調査機関に提出された国際出願の翻訳文に基づき国際調査を行った。

ｂ．この国際出願は、ヌクレオチド又はアミノ酸配列を含んでおり、次の配列表に基づき国際調査を行った。
☐ この国際出願に含まれる書面による配列表
☐ この国際出願と共に提出されたフレキシブルディスクによる配列表
☐ 出願後に、この国際調査機関に提出された書面による配列表
☐ 出願後に、この国際調査機関に提出されたフレキシブルディスクによる配列表
☐ 出願後に提出した書面による配列表が出願時における国際出願の開示の範囲を超える事項を含まない旨の陳述書の提出があった。
☐ 書面による配列表に記載した配列とフレキシブルディスクによる配列表に記録した配列が同一である旨の陳述書の提出があった。

２．☐ 請求の範囲の一部の調査ができない（第Ⅰ欄参照）。

３．☐ 発明の単一性が欠如している（第Ⅱ欄参照）。

４．発明の名称は
☒ 出願人が提出したものを承認する。
☐ 次に示すように国際調査機関が作成した。

５．要約は
☒ 出願人が提出したものを承認する。
☐ 第Ⅲ欄に示されているように、法施行規則第47条（ＰＣＴ規則38.2(b)）の規定により国際調査機関が作成した。出願人は、この国際調査報告の発送の日から１カ月以内にこの国際調査機関に意見を提出することができる。

６．要約書とともに公表される図は、
第 ＿＿＿＿ 図とする。☐ 出願人が示したとおりである。　☒ なし
☐ 出願人は図を示さなかった。
☐ 本図は発明の特徴を一層よく表している。

国際調[redacted]報告　　国際出[redacted]　PCT／JP00／04683

A.　発明の属する分野の分類（国際特許分類（IPC））
Int. Cl⁷ A61K9／52, A61K38／09, A61K47／12, A61K47／34,
A61P35／00, A61P5／24, A61P13／08, A61P15／00

B.　調査を行った分野
調査を行った最小限資料（国際特許分類（IPC））
Int. Cl⁷ A61K9／00－9／72 , A61K47／00－47／48,
A61K38／00－38／58

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）
CA（STN）, REGISTRY（STN）、MEDLINE（STN）, WPI／L（QUESTEL）

C.　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
P,X	WO,99/36099, A1(TAKEDA CHEMICAL INDUSTRIES,LTD.) 22.7月.1999 (22.07.99),明細書全体, 特に特許請求の範囲の記載 & AU,9918897,A & JP,11-269094,A	1-23
A	WO,98/32423, A1(TAKEDA CHEMICAL INDUSTRIES,LTD.) 30.7月.1998 (30.07.98),特許請求の範囲 & AU,9856783,A & JP,10-273447,A	1-23

☒ C欄の続きにも文献が列挙されている。　☐ パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
「E」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」口頭による開示、使用、展示等に言及する文献
「P」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
05．10．00	17.10.00

国際調査機関の名称及びあて先	特許庁審査官（権限のある職員）	4C	3039
日本国特許庁（ISA／JP） 郵便番号100－8915 東京都千代田区霞が関三丁目4番3号	高原　慎太郎		
	電話番号　03－3581－1101　内線　3452		

様式PCT／ISA／210（第2ページ）（1998年7月）

C（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
A	WO,96/22786, A1(TAKEDA CHEMICAL INDUSTRIES,LTD.) 1.8月.1996 (01.08.96),特許請求の範囲 & AU,9644591,A & JP,8-259460,A	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/04683

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K9/52, A61K38/09, A61K47/12, A61K47/34, A61P35/00, A61P5/24, A61P13/08, A61P15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K9/00-9/72 , A61K47/00-47/48, A61K38/00-38/58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN),REGISTY(STN),MEDLINE(STN),WPI/L(QUESTEL)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO, 99/36099, A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 22 July, 1999 (22.07.99), entire specification, , especially claims & AU, 9918897, A & JP, 11-269094, A	1-23
A	WO, 98/32423, A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 30 July, 1998 (30.07.98), Claims & AU, 9856783, A & JP, 10-273447, A	1-23
A	WO, 96/22786, A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 01 August, 1996 (01.08.96), Claims & AU, 9644591, A & JP, 8-259460, A	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 October, 2000 (05.10.00)	17 October, 2000 (17.10.00)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

世界知的所有権機関
国際事務局

PCT

特許協力条約に基づいて公開された国際出願

(51) 国際特許分類6
A61K 47/30, 47/12, 37/02

A1

(11) 国際公開番号 WO99/36099

(43) 国際公開日 1999年7月22日(22.07.99)

(21) 国際出願番号 PCT/JP99/00086

(22) 国際出願日 1999年1月13日(13.01.99)

(30) 優先権データ
特願平10/6412 1998年1月16日(16.01.98) JP

(71) 出願人（米国を除くすべての指定国について）
武田薬品工業株式会社
(TAKEDA CHEMICAL INDUSTRIES, LTD.)[JP/JP]
〒541-0045 大阪府大阪市中央区道修町四丁目1番1号
Osaka, (JP)
(72) 発明者；および
(75) 発明者／出願人（米国についてのみ）
犀川 彰(SAIKAWA, Akira)[JP/JP]
〒617-0823 京都府長岡京市長岡2丁目2番45号 岩田ビル3F
Kyoto, (JP)
猪狩康孝(IGARI, Yasutaka)[JP/JP]
〒658-0015 兵庫県神戸市東灘区本山南町5丁目4番25-503号
Hyogo, (JP)
畑 善夫(HATA, Yoshio)[JP/JP]
〒560-0001 大阪府豊中市北緑丘2丁目1番12-703号 Osaka, (JP)
山本一路(YAMAMOTO, Kazumichio)[JP/JP]
〒631-0033 奈良県奈良市あやめ池南1丁目7番10-116号
Nara, (JP)
(74) 代理人
弁理士 朝日奈忠夫, 外(ASAHINA, Tadao et al.)
〒532-0024 大阪府大阪市淀川区十三本町2丁目17番85号
武田薬品工業株式会社 大阪工場内 Osaka, (JP)

(81) 指定国 AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO特許 (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), ユーラシア特許 (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), 欧州特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI特許 (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

添付公開書類
国際調査報告書
請求の範囲の補正の期限前の公開；補正書受領の際には再公開される。

(54)Title: SUSTAINED RELEASE COMPOSITIONS, PROCESS FOR PRODUCING THE SAME AND UTILIZATION THEREOF

(54)発明の名称 徐放性組成物、その製造法および用途

(57) Abstract
Sustained release compositions containing a physiologically active substance or its salt, hydroxynaphthoic acid or its salt and a biodegradable polymer or its salt; and drugs, etc. containing these compositions.

(57)要約

生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩を含有してなる徐放性組成物その製造法および該徐放性組成物を含有する医薬などに関する。

明 細 書

徐放性組成物、その製造法および用途

技術分野

本発明は、生理活性物質の徐放性製剤およびその製造法に関する。

背景技術

特開平７－９７３３４号公報には、生理活性ペプチドまたはその塩と末端に遊離のカルボキシル基を有する生体内分解性ポリマーとからなる徐放性製剤およびその製造法が開示されている。

ＧＢ２２０９９３７号、ＧＢ２２３４１６９号、ＧＢ２２３４８９６号、ＧＢ２２５７９０９号公報およびＥＰ６２６１７０Ａ２号公報には、別途調製したペプチド、タンパク質のパモ酸塩等の水不溶性塩を含んでなる生体内分解性ポリマーを基剤とした組成物またはその製造法が開示されている。

ＷＯ９５／１５７６７号公報には、cetrorelix（LH－RHアンタゴニスト）のエンボン酸塩（パモ酸塩）およびその製造法が開示されていると同時に、このパモ酸塩を生体内分解性ポリマーに封入してもそのペプチドの放出性はパモ酸塩単独での場合と同様であることが記述されている。

発明の開示

生理活性物質を高含量で含有し、かつその初期過剰放出を抑制して長期にわたる安定した放出速度を実現できる新規組成物を提供する。

本発明者らは、上記の問題点を解決するために鋭意研究の結果、組成物を形成させる際に生理活性物質とヒドロキシナフトエ酸を共存させることにより生理活性物質を高含量で組成物中に取り込み、さらに生体内分解性ポリマー中に

両者を封入した場合は、生体内分解性ポリマーが存在しない条件下で調製した生理活性物質とヒドロキシナフトエ酸から形成される組成物からの生理活性物質の放出速度とは異なる速度で生理活性物質が放出され、その放出速度が生体内分解性ポリマーの特性やヒドロキシナフトエ酸の添加量によって制御可能であり、高含量においても確実に初期過剰放出を抑制して、非常な長期にわたる持続放出を実現させることができ、さらに研究を重ねた結果、本発明を完成するに至った。

すなわち、本発明は、

（１）生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩を含有してなる徐放性組成物、

（２）生理活性物質が生理活性ペプチドである第（１）項記載の徐放性組成物、

（３）生理活性物質がLH-RH誘導体である第（２）項記載の徐放性組成物。

（４）ヒドロキシナフトエ酸が３－ヒドロキシー２－ナフトエ酸である第（１）項記載の徐放性組成物、

（５）生体内分解性ポリマーがα－ヒドロキシカルボン酸重合体である第（１）項記載の徐放性組成物、

（６）α－ヒドロキシカルボン酸重合体が乳酸－グリコール酸重合体である第（５）項記載の徐放性組成物、

（７）乳酸とグリコール酸の組成モル％が１００／０～４０／６０である第（６）項記載の徐放性組成物、

（８）乳酸とグリコール酸の組成モル％が１００／０である第（７）項記載の徐放性組成物、

（９）重合体の重量平均分子量が約３，０００～約１００，０００である第（６）項記載の徐放性組成物、

（１０）重量平均分子量が約２０，０００～５０，０００である第（９）項記載の徐放性組成物、

（１１）LH-RH 誘導体が式

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z

[式中、Y は DLeu、DAla、DTrp、DSer(tBu)、D2Nal または DHis(ImBzl)を示し、Z は NH-C_2H_5 または Gly-NH_2 を示す。]で表されるペプチドである第（３）項記載の徐放性組成物、

（１２）重合体の末端のカルボキシル基量が重合体の単位質量（グラム）あたり５０－９０マイクロモルである第（６）項記載の徐放性組成物、

（１３）ヒドロキシナフトエ酸またはその塩と LH-RH 誘導体またはその塩のモル比が３対４ないし４対３である第（３）項記載の徐放性組成物、

（１４）徐放性組成物中、LH-RH 誘導体またはその塩が１４％(w/w)から２４％(w/w)含有される第（１３）項記載の徐放性組成物、

（１５）生理活性物質またはその塩が微水溶性または水溶性である第（１）項記載の徐放性組成物、

（１６）注射用である第（１）項記載の徐放性組成物、

（１７）生理活性物質またはその塩、生体内分解性ポリマーまたはその塩およびヒドロキシナフトエ酸またはその塩の混合液から溶媒を除去することを特徴とする第（１）項記載の徐放性組成物の製造法、

（１８）生体内分解性ポリマーまたはその塩およびヒドロキシナフトエ酸またはその塩を含有する有機溶媒溶液に生理活性物質またはその塩を混合、分散し、次いで有機溶媒を除去することを特徴とする第（１７）項記載の徐放性組成物の製造法、

（１９）生理活性物質またはその塩が生理活性物質またはその塩を含有する水溶液である第（１８）項記載の徐放性組成物の製造法、

（２０）生理活性物質の塩が遊離塩基または酸との塩である第（１７）項記載の製造法、

（２１）第（１）項記載の徐放性組成物を含有してなる医薬、

（２２）第（３）項記載の徐放性組成物を含有してなる前立腺癌、前立腺肥大症、子宮内膜症、子宮筋腫、子宮線維腫、思春期早発症、月経困難症もしくは乳癌の予防、治療剤または避妊剤、

（２３）生理活性物質のヒドロキシナフトエ酸塩および生体内分解性ポリマーまたはその塩を含有してなる徐放性組成物、

（２４）ヒドロキシナフトエ酸またはその塩を用いることを特徴とする徐放性組成物からの生理活性物質の初期過剰放出を抑制する方法、

（２５）ヒドロキシナフトエ酸またはその塩を用いることを特徴とする徐放性組成物への生理活性物質の封入効率を向上する方法、

（２６）生理活性ペプチドのヒドロキシナフトエ酸塩、

（２７）水溶性または微水溶性である第（２６）項記載の生理活性ペプチドのヒドロキシナフトエ酸塩、および

（２８）生理活性ペプチドのヒドロキシナフトエ酸塩を含有してなる徐放性組成物などを提供する。

さらに、本発明は、

（２９）ヒドロキシナフトエ酸またはその塩の配合量が生理活性ペプチドまたはその塩１モルに対して約１～約７モル、好ましくは約１～約２モルである第（２８）項記載の徐放性組成物、

（３０）生理活性物質またはその塩を含む液を内水相とし、生体内分解性ポリマーおよびヒドロキシナフトエ酸またはその塩を含む溶液を油相とするW／O型乳化物を製造し、次いで溶媒を除去することを特徴とする第（１７）項記載の徐放性組成物の製造法、

（３１）ヒドロキシナフトエ酸またはその塩を含む液を内水相とし、生理活性物質またはその塩および生体内分解性ポリマーまたはその塩を含む溶液を油相とするW／O型乳化物を製造し、次いで溶媒を除去することを特徴とする第（１７）項記載の徐放性組成物の製造法、

（３２）生理活性ペプチドまたはその塩およびヒドロキシナフトエ酸またはその塩を混合、溶解し、次いで溶媒を除去することを特徴とする第（２８）項記載の徐放性組成物の製造法、

および

（３３）溶媒の除去法が水中乾燥法である第（３０）項～第（３２）項のいずれかに記載の徐放性組成物の製造法などを提供する。

本発明で用いられる生理活性物質は、薬理学的に有用なものであれば特に限定を受けないが、非ペプチド化合物でもペプチド化合物でもよい。非ペプチド化合物としては、アゴニスト、アンタゴニスト、酵素阻害作用を有する化合物などがあげられる。また、ペプチド化合物としては、例えば、生理活性ペプチドが好ましく、分子量約３００～約４０，０００、好ましくは約４００～約３０，０００、さらに好ましくは約５００～約２０，０００の生理活性ペプチドなどが好適である

該生理活性ペプチドとしては、例えば、黄体形成ホルモン放出ホルモン（ＬＨ－ＲＨ）、インスリン、ソマトスタチン、成長ホルモン、成長ホルモン放出ホルモン(ＧＨ－ＲＨ)、プロラクチン、エリスロポイエチン、副腎皮質ホルモン、メラノサイト刺激ホルモン、甲状腺ホルモン放出ホルモン、甲状腺刺激ホルモン、黄体形成ホルモン、卵胞刺激ホルモン、バソプレシン、オキシトシン、カルシトニン、ガストリン、セクレチン、パンクレオザイミン、コレシストキニン、アンジオテンシン、ヒト胎盤ラクトーゲン、ヒト絨毛性ゴナドトロピン、エンケファリン、エンドルフィン、キョウトルフィン、タフトシン、サイモポイエチン、サイモシン、サイモチムリン、胸腺液性因子、血中胸腺因子、腫瘍壊死因子、コロニー誘導因子、モチリン、デイノルフィン、ボンベシン、ニューロテンシン、セルレイン、ブラジキニン、心房性ナトリウム排泄増加因子、神経成長因子、細胞増殖因子、神経栄養因子、エンドセリン拮抗作用を有する

ペプチド類などおよびその誘導体、さらにはこれらのフラグメントまたはフラグメントの誘導体などが挙げられる。

本発明で用いられる生理活性物質はそれ自身であっても、薬理学的に許容される塩であってもよい。

このような塩としては、該生理活性物質がアミノ基等の塩基性基を有する場合、無機酸（無機の遊離酸とも称する）（例、炭酸、重炭酸、塩酸、硫酸、硝酸、ホウ酸等）、有機酸（有機の遊離酸とも称する）（例、コハク酸、酢酸、プロピオン酸、トリフルオロ酢酸等）などとの塩が挙げられる。

生理活性物質がカルボキシル基等の酸性基を有する場合、無機塩基（無機の遊離塩基とも称する）（例、ナトリウム、カリウム等のアルカリ金属、カルシウム、マグネシウム等のアルカリ土類金属など）や有機塩基（有機の遊離塩基とも称する）（例、トリエチルアミン等の有機アミン類、アルギニン等の塩基性アミノ酸類等）などとの塩が挙げられる。また、生理活性ペプチドは金属錯体化合物（例、銅錯体、亜鉛錯体等）を形成していてもよい。

該生理活性ペプチドの好ましい例としては、ＬＨ－ＲＨ誘導体であって、ホルモン依存性疾患、特に性ホルモン依存性癌（例、前立腺癌、子宮癌、乳癌、下垂体腫瘍など）、前立腺肥大症、子宮内膜症、子宮筋腫、思春期早発症、月経困難症、無月経症、月経前症候群、多房性卵巣症候群等の性ホルモン依存性の疾患および避妊（もしくは、その休薬後のリバウンド効果を利用した場合には、不妊症）に有効なＬＨ－ＲＨ誘導体またはその塩が挙げられる。さらに性ホルモン非依存性であるがＬＨ－ＲＨ感受性である良性または悪性腫瘍などに有効なＬＨ－ＲＨ誘導体またはその塩も挙げられる。

ＬＨ－ＲＨ誘導体またはその塩の具体例としては、例えば、トリートメントウイズ　ＧｎＲＨ　アナログ：コントラバーシス　アンド　パースペクテイブ（Treatment with GnRH analogs: Controversies and perspectives）［パルテノン　パブリッシング　グループ（株）（The Parthenon Publishing Group Ltd.）

発行1996年〕、特表平３－５０３１６５号公報、特開平３－１０１６９５号、同７－９７３３４号および同８－２５９４６０号公報などに記載されているペプチド類が挙げられる。

ＬＨ－ＲＨ誘導体としては、ＬＨ－ＲＨアゴニストまたはＬＨ－ＲＨアンタゴニストが挙げられるが、ＬＨ－ＲＨアンタゴニストとしては、例えば、一般式〔Ｉ〕

X-D2Nal-D4ClPhe-D3Pal-Ser-A-B-Leu-C-Pro-DAlaNH$_2$

〔式中、ＸはN(4H$_2$-furoyl)GlyまたはNAcを、ＡはNMeTyr、Tyr、Aph(Atz)、NMeAph(Atz)から選ばれる残基を、ＢはDLys(Nic)、DCit、DLys(AzaglyNic)、DLys(AzaglyFur)、DhArg(Et$_2$)、DAph(Atz)およびDhCiから選ばれる残基を、ＣはLys(Nisp)、ArgまたはhArg(Et$_2$)をそれぞれ示す〕で表わされる生理活性ペプチドまたはその塩などが用いられる。

ＬＨ－ＲＨアゴニストとしては、例えば、一般式〔II〕

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z

〔式中、ＹはDLeu、DAla、DTrp、DSer(tBu)、D2NalおよびDHis(ImBzl)から選ばれる残基を、ＺはNH-C$_2$H$_5$またはGly-NH$_2$をそれぞれ示す〕で表わされる生理活性ペプチドまたはその塩などが用いられる。特に、ＹがDLeuで、ＺがNH-C$_2$H$_5$であるペプチド（即ち、5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C$_2$H$_5$で表されるペプチド）が好適である。

これらのペプチドは、前記文献あるいは公報記載の方法あるいはこれに準じる方法で製造することができる。

本明細書中で使用される略号の意味は次のとおりである。

略号　　名称

N(4H$_2$-furoyl)Gly：　N-テトラヒドロフロイルグリシン残基

NAc：　N-アセチル基

D2Nal：　D-3-(2-ナフチル）アラニン残基

D4ClPhe： D-3-(4-クロロ) フェニルアラニン残基

D3Pal： D-3-(3-ピリジル) アラニン残基

NMeTyr： N-メチルチロシン残基

Aph(Atz)： N-[5'-(3'-アミノ-1'H-1', 2', 4'-トリアゾリル)]フェニルアラニン残基

NMeAph(Atz)： N-メチル-[5'-(3'-アミノ-1'H-1', 2', 4'-トリアゾリル)]フェニルアラニン残基

DLys(Nic)： D-(e-N-ニコチノイル) リシン残基

Dcit： D-シトルリン残基

DLys(AzaglyNic)： D-(アザグリシルニコチノイル) リシン残基

DLys(AzaglyFur)： D-(アザグリシルフラニル) リシン残基

DhArg(Et_2)： D-(N, N'-ジエチル)ホモアルギニン残基

DAph(Atz)： D-N-[5'-(3'-アミノ-1'H-1', 2', 4'-トリアゾリル)]フェニルアラニン残基

DhCi： D-ホモシトルリン残基

Lys(Nisp)： (e-N-イソプロピル) リシン残基

hArg(Et_2)： (N, N'-ジエチル)ホモアルギニン残基

その他アミノ酸に関し、略号で表示する場合、IUPAC-IUB コミッション・オブ・バイオケミカル・ノーメンクレーチュアー(Commission on Biochemical Nomenclature) (ヨーロピアン・ジャーナル・オブ・バイオケミストリー(European Journal of Biochemistry)第138巻、9～37頁 (1984年)) による略号または該当分野における慣用略号に基づくものとし、また、アミノ酸に関して光学異性体がありうる場合は、特に明示しなければL体を示すものとする。

本発明に用いられるヒドロキシナフトエ酸は、ナフタレンの異なる炭素に1つの水酸基と1つのカルボキシル基が結合したものである。従って、カルボキシ

ル基の位置がナフタレン環の１位と２位であるそれぞれに対して水酸基の位置が異なる合計１４種の異性体が存在する。そしてこの中の任意の異性体を用いてよく、またこれらの任意の割合の混合物を用いてもよい。後述するが、酸解離定数の大きなものが好ましく、あるいはｐＫａ（ｐＫａ＝－ｌｏｇ$_{10}$Ｋａ、Ｋａは酸解離定数を表す）の小さいものが好ましい。そして微水溶性のものが好ましい。

また、アルコール類（例えば、エタノール、メタノール等）に可溶であるものが好ましい。「アルコール類に可溶」とは例えばメタノールに対して１０ｇ／Ｌ以上であることを意味する。

上記のヒドロキシナフトエ酸異性体のｐＫａとしては、３－ヒドロキシ－２－ナフトエ酸の値（ｐＫａ＝２．７０８、化学便覧　基礎編Ⅱ、日本化学会、昭和４４年９月２５日発行）のみが知られているが、ヒドロキシ安息香酸の３種の異性体のｐＫａを比較することによって有用な知見が得られる。すなわちｍ－ヒドロキシ安息香酸とｐ－ヒドロキシ安息香酸のｐＫａが４以上であるのに対してｏ－ヒドロキシ安息香酸（サリチル酸）のｐＫａ（＝２．７５４）は極端に小さい。従って、上記１４種の異性体のなかでも、ナフタレン環の隣接する炭素原子にカルボキシル基と水酸基が結合した、３－ヒドロキシ－２－ナフトエ酸、１－ヒドロキシ－２－ナフトエ酸および２－ヒドロキシ－１－ナフトエ酸が好ましい。さらには、ナフタレンの３位の炭素に水酸基が、２位の炭素にカルボキシル基が結合した３－ヒドロキシ－２－ナフトエ酸が好適である。

ヒドロキシナフトエ酸は塩であってもよい。塩としては、例えば、無機塩基（例、ナトリウム、カリウム等のアルカリ金属、カルシウム、マグネシウム等のアルカリ土類金属など）や有機塩基（例、トリエチルアミン等の有機アミン類、アルギニン等の塩基性アミノ酸類等）などとの塩、または遷移金属（例，亜鉛，鉄，銅など）との塩および錯塩などが挙げられる。

以下に、本発明の生理活性物質のヒドロキシナフトエ酸塩の調製方法を例示する。

（１）ヒドロキシナフトエ酸の含水有機溶媒溶液を弱塩基性イオン交換カラムに

通して吸着させ、そして飽和させる。次いで含水有機溶媒を通して過剰のヒドロキシナフトエ酸を除去した後に生理活性物質またはその塩の含水有機溶媒溶液を通してイオン交換を行わせて、得られた流出液から溶媒を除去すればよい。該含水有機溶媒中の有機溶媒としては、アルコール類（例、メタノール、エタノール等）、アセトニトリル、テトラヒドロフラン、ジメチルホルムアミドなどが用いられる。塩を析出させるための溶媒を除去する方法は、自体公知の方法あるいはそれに準じる方法が用いられる。例えば、ロータリーエヴァポレーターなどを用いて真空度を調節しながら溶媒を蒸発させる方法などが挙げられる。

（2）予め、強塩基性イオン交換カラムの交換イオンを水酸化物イオンに交換しておき、これに生理活性物質またはその塩の含水有機溶媒溶液を通してそれらの塩基性基を水酸化型に換える。回収した流出液に当量以下のヒドロキシナフトエ酸を加えて溶解し、次いで濃縮して析出した塩を、必要な場合には水洗して、乾燥すればよい。

生理活性物質のヒドロキシナフトエ酸塩は、用いる生理活性物質にもよるが、微水微溶性であるため、特に生理活性ペプチドの該塩自身が徐放能を発揮して生理活性物質の徐放性製剤に用いることができるし、また、さらに徐放性組成物を製造することもできる。

本発明に用いられる生体内分解性ポリマーとしては、例えば、α－ヒドロキシモノカルボン酸類（例、グリコール酸、乳酸等）、α－ヒドロキシジカルボン酸類（例、リンゴ酸）、α－ヒドロキシトリカルボン酸（例、クエン酸）等のα－ヒドロキシカルボン酸類の1種以上から合成され、遊離のカルボキシル基を有する重合体、共重合体、またはこれらの混合物；ポリ（α－シアノアクリル酸エステル）；ポリアミノ酸（例、ポリ（γ－ベンジル－L－グルタミン酸）等）；無水マレイン酸系共重合体（例、スチレン－マレイン酸共重合体等）などが用いられる。

モノマーの結合様式としては、ランダム、ブロック、グラフトのいずれでもよい。また、上記α－ヒドロキシモノカルボン酸類、α－ヒドロキシジカルボン酸類、α－ヒドロキシトリカルボン酸類が分子内に光学活性中心を有する場

合、Ｄ－、Ｌ－、ＤＬ－体のいずれを用いてもよい。これらの中でも、乳酸－グリコール酸重合体（以下、ポリ（ラクチド－ｃｏ－グリコリド）、ポリ（乳酸－ｃｏ－グリコール酸）あるいは乳酸－グリコール酸共重合体と称することもあり、特に明示しない限り、乳酸、グリコール酸のホモポリマー（重合体）及びコポリマー（共重合体）を総称する。また乳酸ホモポリマーは乳酸重合体、ポリ乳酸、ポリラクチドなどと、またグリコール酸ホモポリマーはグリコール酸重合体、ポリグリコール酸、ポリグリコリドなどと称される場合がある）、ポリ（α－シアノアクリル酸エステル）などが好ましい。さらに好ましくは、乳酸－グリコール酸重合体であり、より好ましくは、末端に遊離のカルボキシル基を有する乳酸－グリコール酸重合体である。

生体内分解性ポリマーは塩であってもよい。塩としては、例えば、無機塩基（例、ナトリウム、カリウム等のアルカリ金属、カルシウム、マグネシウム等のアルカリ土類金属など）や有機塩基（例、トリエチルアミン等の有機アミン類、アルギニン等の塩基性アミノ酸類等）などとの塩、または遷移金属（例，亜鉛，鉄，銅など）との塩および錯塩などが挙げられる。

生体内分解性ポリマーとして乳酸－グリコール酸重合体を用いる場合、その組成比（モル％）は約１００／０～約４０／６０が好ましく、約１００／０～約５０／５０がより好ましい。また、組成比が１００／０である乳酸ホモポリマーも好ましく用いられる。

該「乳酸－グリコール酸重合体」の最小繰り返し単位の一つである乳酸の光学異性体比は、Ｄ－体／Ｌ－体（モル／モル％）が約７５／２５～約２５／７５の範囲のものが好ましい。このＤ－体／Ｌ－体（モル／モル％）は、特に約６０／４０～約３０／７０の範囲のものが汎用される。

該「乳酸－グリコール酸重合体」の重量平均分子量は、通常、約３，０００～約１００，０００、好ましくは約３，０００～約６０，０００、さらに好ましくは約３，０００～約５０，０００、特に好ましくは約２０，０００～約５

０，０００のものが用いられる。

また、分散度（重量平均分子量／数平均分子量）は、通常約１．２～約４．０が好ましく、さらには約１．５～３．５が特に好ましい。

該「乳酸－グリコール酸重合体」の遊離のカルボキシル基量は、重合体の単位質量（グラム）あたり通常約２０～約１０００μ mol（マイクロモル）が好ましく、さらには約４０～約１０００μ mol（マイクロモル）が特に好ましい。

本明細書における重量平均分子量、数平均分子量および分散度とは、重量平均分子量が１，１１０，０００、７０７，０００、４５５，６４５、３５４，０００、１８９，０００、１５６，０５５、９８，９００、６６，４３７、３７，２００、１７，１００、９，８３０、５，８７０、２，５００、１，３０３、５０４の１５種類の単分散ポリスチレンを基準物質としてゲルパーミエーションクロマトグラフィー（ＧＰＣ）で測定したポリスチレン換算の分子量および算出した分散度をいう。測定は、高速ＧＰＣ装置（東ソー製、ＨＬＣ－８１２０ＧＰＣ、検出方式は示差屈折率による）、ＧＰＣカラムＫＦ８０４Ｌ×２（昭和電工製）を使用し、移動相としてクロロホルムを用いる。流速は１ｍｌ／ｍｉｎでおこなう。

本明細書における遊離のカルボキシル基量とはラベル化法により求めたもの（以下、「ラベル化法によるカルボキシル基量」と称する）をいう。具体的にポリ乳酸の場合について述べると、ポリ乳酸　Ｗｍｇを５Ｎ塩酸／アセトニトリル（ｖ／ｖ＝４／９６）混液２ｍｌに溶解し、０．０１Ｍ　ｏ－ニトロフェニルヒドラジン塩酸塩（ＯＮＰＨ）溶液（５Ｎ塩酸／アセトニトリル／エタノール＝１．０２／３５／１５）２ｍｌと０．１５Ｍ　１－エチル－３－（３－ジメチルアミノプロピル）－カルボジイミド塩酸塩溶液（ピリジン／エタノール＝４ｖ／９６ｖ）２ｍｌを加えて４０℃で３０分反応させた後溶媒を留去する。残滓を水洗（４回）した後、アセトニトリル２ｍｌで溶解し、０．５ｍｏｌ／ｌのエタノール性水酸化カリウム溶液１ｍｌを加えて６０℃で３０分反応

させる。反応液を１．５Ｎ水酸化ナトリウム水溶液で希釈してＹｍｌとし、１．５Ｎ水酸化ナトリウム水溶液を対象として５４４ｎｍ吸光度Ａ（／ｃｍ）を測定する。一方、ＤＬ－乳酸水溶液を基準物質として、その遊離カルボキシル基量　Ｃｍｏｌ／Ｌをアルカリ滴定で求め、またＯＮＰＨラベル化法でＤＬ－乳酸ヒドラジドとしたときの５４４ｎｍ吸光度を　Ｂ（／ｃｍ）とするとき、重合体の単位質量（グラム）あたりの遊離のカルボキシル基のモル量は以下の数式で求められる。

［ＣＯＯＨ］（ｍｏｌ／ｇ）＝（ＡＹＣ）／（ＷＢ）

また、該「カルボキシル基量」は生体内分解性ポリマーをトルエン－アセトン－メタノール混合溶媒に溶解し、フェノールフタレインを指示薬としてこの溶液をアルコール性水酸化カリウム溶液でカルボキシル基を滴定して求めることもできる（以下、この方法によって求めた値を「アルカリ滴定法によるカルボキシル基量」と称する）が、滴定中にポリエステル主鎖の加水分解反応を競合する結果、滴定終点が不明確になる可能性があり上記ラベル化法で定量するのが望ましい。

生体内分解性ポリマーの分解・消失速度は共重合組成、分子量あるいは遊離カルボキシル基量によって大きく変化するが、乳酸-グリコール酸重合体の場合、一般的にはグリコール酸分率が低いほど分解・消失が遅いため、グリコール酸分率を低くするかあるいは分子量を大きくし、かつ遊離カルボキシル基量を少なくすることによって放出期間を長くすることができる。しかし、遊離カルボキシル基量は生理活性物質の製剤への取り込み率に影響するので一定値以上必要である。この故に、長期間（例えば、６カ月以上）型徐放性製剤用の生体内分解性ポリマーとするには、乳酸-グリコール酸重合体の場合、上記の重量平均分子量が約２０，０００～約５０，０００で、かつ遊離カルボキシル基量が約３０～約９５μｍｏｌ／ｇ、好ましくは約４０～約９５μｍｏｌ／ｇ、より好ましくは約５０～約９０μｍｏｌ／ｇであるポリ乳酸（例、Ｄ－乳酸、Ｌ－乳

酸、ＤＬ－乳酸など、特にＤＬ－乳酸などが好ましい）が好ましい。

該「乳酸－グリコール酸重合体」は、例えば、乳酸とグリコール酸からの無触媒脱水重縮合（特開昭６１－２８５２１号）あるいはラクチドとグリコリド等の環状ジエステル化合物からの触媒を用いた開環重合（Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials, Volume 2, Marcel Dekker, Inc. 1995 年）で製造できる。上記の公知の開環重合方法によって得られる重合体は、得られる重合体の末端に遊離のカルボキシル基を有しているとは限らないが、例えば、ＥＰ－Ａ－０８３９５２５号に記載の加水分解反応に付すことにより、単位質量当たりにある程度のカルボキシル基量を有する重合体に改変することができ、これを用いることもできる。

上記の「末端に遊離のカルボキシル基を有する乳酸－グリコール酸重合体」は公知の製造法（例えば無触媒脱水重縮合法、特開昭６１－２８５２１号公報参照）で問題なく製造でき、あるいは、下記の方法によっても製造できる。

（１）まず、カルボキシル基が保護されたヒドロキシモノカルボン酸誘導体（例、Ｄ－乳酸 tert-ブチル、Ｌ－乳酸ベンジルなど）またはカルボキシル基が保護されたヒドロキシジカルボン酸誘導体（例、タルトロン酸ジベンジル、２－ヒドロキシエチルマロン酸ジ tert-ブチルなど）の存在下、重合触媒を用いて環状エステル化合物を重合反応に付す。

上記の「カルボキシル基が保護されたヒドロキシモノカルボン酸誘導体」または「カルボキシル基が保護されたヒドロキシジカルボン酸誘導体」とは、例えば、カルボキシル基（－ＣＯＯＨ）がアミド（－$CONH_2$）化またはエステル（－ＣＯＯＲ）化されているヒドロキシカルボン酸誘導体などがあげられるが、なかでも、カルボキシル基（－ＣＯＯＨ）がエステル（－ＣＯＯＲ）化されているヒドロキシカルボン酸誘導体などが好ましい。

ここでエステルにおけるＲとしては、例えば、メチル、エチル、ｎ－プロピル、イソプロピル、ｎ－ブチル、tert－ブチルなどのC_{1-6}アルキル基、例え

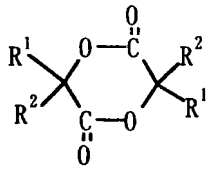
ば、シクロペンチル、シクロヘキシルなどのC_{3-8}シクロアルキル基、例えば、フェニル、α－ナフチルなどのC_{6-12}アリール基、例えば、ベンジル、フェネチルなどのフェニル－C_{1-2}アルキル基もしくはα－ナフチルメチルなどのα－ナフチル－C_{1-2}アルキル基などのC_{7-14}アラルキル基などがあげられる。なかでも、tert－ブチル基、ベンジル基などが好ましい。

該「環状エステル化合物」とは、例えば環内に少なくとも１つのエステル結合を有する環状化合物をいう。具体的には、環状モノエステル化合物（ラクトン類）または環状ジエステル化合物（ラクチド類）などがあげられる。

該「環状モノエステル化合物」としては、例えば、４員環ラクトン（β－プロピオラクトン、β－ブチロラクトン、β－イソバレロラクトン、β－カプロラクトン、β－イソカプロラクトン、β－メチル－β－バレロラクトンなど）、５員環ラクトン（γ－ブチロラクトン、γ－バレロラクトンなど）、６員環ラクトン（δ－バレロラクトンなど）、７員環ラクトン（ε－カプロラクトンなど）、p-ジオキサノン、1,5-ジオキセパン－２－オンなどがあげられる。

該「環状ジエステル化合物」としては、

例えば、式



（式中、R^1およびR^2はそれぞれ同一または異なって、水素原子またはメチル、エチル、ｎ－プロピル、イソプロピル、ｎ－ブチル、ｔ－ブチルなどのC_{1-6}アルキル基を示す）で表される化合物などがあげられ、なかでも、R^1が水素原子でR^2がメチル基、R^1およびR^2がそれぞれ水素原子であるラクチドなどが好ましい。

具体的には、たとえばグリコリド、L-ラクチド、D-ラクチド、DL-ラクチド、

meso-ラクチド、3-メチル-1,4-ジオキサン-2,5-ジオン（光学活性体も含む）などがあげられる。

該「重合触媒」としては、例えば有機スズ系触媒（例、オクチル酸スズ、ジラウリル酸ジーｎ－ブチルスズ、テトラフェニルスズなど）、アルミ系触媒（例、トリエチルアルミニウムなど）、亜鉛系触媒（例、ジエチル亜鉛など）などがあげられる。

反応後の除去の容易さの観点からは、アルミ系触媒、亜鉛系触媒が好ましく、さらには、残存した場合の安全性の観点からは亜鉛系触媒が好ましい。

重合触媒の溶媒としては、ベンゼン、ヘキサン、トルエンなどが用いられ、中でもヘキサン、トルエンなどが好ましい。

「重合方法」は、反応物を融解状態にして行う塊状重合法または反応物を適当な溶媒（例えば、ベンゼン、トルエン、キシレン、デカリン、ジメチルホルムアミドなど）に溶解して行う溶液重合法を用いればよい。溶媒としては、トルエン、キシレンなどが好ましい。重合温度は特に限定されるものではないが、塊状重合の場合、反応開始時に反応物を融解状態に至らしめる温度以上、通常１００～３００℃であり、溶液重合の場合、通常室温～１５０℃であり、反応温度が反応溶液の沸点を越えるときは、凝縮器を付けて還流するか、または耐圧容器内で反応させればよい。重合時間は重合温度、そのほかの反応条件や目的とする重合体の物性などを考慮して適宜定められるが、例えば１０分～７２時間である。反応後は、必要であれば反応混合物を適当な溶媒（例えば、アセトン、ジクロロメタン、クロロホルムなど）に溶解し、酸（例えば、塩酸、無水酢酸、トリフルオロ酢酸など）で重合を停止させた後、常法によりこれを目的物を溶解しない溶媒（例えば、アルコール、水、エーテル、イソプロピルエーテルなど）中に混合するなどして析出させ、ω端に保護されたカルボキシル基を有するポリマーを単離すればよい。

本願の重合方法は、従来のメタノールなどのいわゆるプロトン性連鎖移動剤

の代わりにカルボキシル基が保護されたヒドロキシカルボン酸誘導体（例、Ｄ－乳酸 tert-ブチル、Ｌ－乳酸ベンジルなど）またはカルボキシル基が保護されたヒドロキシジカルボン酸誘導体（例、タルトロン酸ジベンジル、２－ヒドロキシエチルマロン酸ジ tert-ブチルなど）などが用いられる。

このようにカルボキシル基が保護されたヒドロキシカルボン酸誘導体（例、Ｄ－乳酸 tert-ブチル、Ｌ－乳酸ベンジルなど）またはカルボキシル基が保護されたヒドロキシジカルボン酸誘導体（例、タルトロン酸ジベンジル、２－ヒドロキシエチルマロン酸ジ tert-ブチルなど）などをプロトン性連鎖移動剤に用いることによって、①分子量を仕込み組成によって制御でき、②重合後に脱保護反応に付すことによって、得られる生体内分解性ポリマーのω端にカルボキシル基を遊離させることができる。

（２）次に、上記（１）の重合反応によって得られたω端に保護されたカルボキシル基を有するポリマーを脱保護反応に付すことにより目的とするω端に遊離のカルボキシル基を有する生体内分解性ポリマーを得ることができる。

該保護基は自体公知の方法により脱離できる。このような方法としては、ポリ（ヒドロキシカルボン酸）のエステル結合に影響を与えずに保護基を除去することが可能な方法であればいずれを用いてもよいが、具体的には、例えば還元、酸分解などの方法が挙げられる。

該還元方法としては、例えば触媒（例、パラジウム炭素、パラジウム黒、酸化白金など）を用いる接触還元、液体アンモニウム中でのナトリウムによる還元、ジチオスレイトールによる還元などが挙げられる。例えば、ω端にベンジル基で保護されたカルボキシル基を有するポリマーを接触還元する場合、具体的にはポリマーを酢酸エチル、ジクロロメタン、クロロホルムなどに溶解したものにパラジウム炭素を添加し、激しく撹拌しながら室温で水素を約２０分～約４時間通気することで脱保護できる。

酸分解方法としては、例えば無機酸（例、フッ化水素、臭化水素、塩化水素

など）あるいは有機酸（例、トリフルオロ酢酸、メタンスルホン酸、トリフルオロメタンスルホン酸など）またはこれらの混合物などによる酸分解などが挙げられる。また、必要に応じて、酸分解の際、カチオン・スカベンジャー（例、アニソール、フェノール、チオアニソールなど）を適宜添加してもよい。例えば、ω端に tert-ブチル基で保護されたカルボキシル基を有するポリマーを酸分解する場合、具体的にはポリマーをジクロロメタン、キシレン、トルエンなどに溶解したものにトリフルオロ酢酸を適当量加えて、あるいはポリマーをトリフルオロ酢酸で溶解して室温で約１時間攪拌することで脱保護できる。

好ましくは、該酸分解法は重合反応直後に行ってもよく、その場合は重合停止反応を兼ねることができる。

さらに必要に応じて、上記の脱保護反応によって得られたポリマーを酸加水分解反応に付すことにより、該ポリマーの重量平均分子量、数平均分子量あるいは末端カルボキシル基量を目的に応じて調節することができる。具体的には、例えば、ＥＰ－Ａ－０８３９５２５号に記載の方法またはそれに準じた方法によって行うことができる。

前記のようにして得られた生体内分解性ポリマーは、徐放性製剤を製造するための基剤として用いることができる。

さらには末端に特定されない遊離のカルボキシル基を有する重合体は公知の製造法（例えば、ＷＯ９４／１５５８７号公報参照）で製造できる。

また、開環重合後の化学的処理によって末端を遊離のカルボキシル基にした乳酸－グリコール酸重合体は例えばベーリンガー　インゲルハイム（Boehringer Ingelheim KG）から市販されているものを用いてもよい。

生体内分解性ポリマーは塩（生体内分解性ポリマーの塩としては例えば前述の塩などがあげられる）であってもよく、その製造方法としては、例えば、（ａ）上記のカルボキシル基を有する生体内分解性ポリマーを有機溶媒に溶解したものと無機塩基（例、ナトリウム、カリウム等のアルカリ金属、カルシウム、マ

グネシウム等のアルカリ土類金属など）や有機塩基（例、トリエチルアミン等の有機アミン類、アルギニン等の塩基性アミノ酸類等）のイオンを含む水溶液を混合してイオン交換反応を行わせた後に、塩となったポリマーを単離する、（b）上記のカルボキシル基を有する生体内分解性ポリマーを有機溶媒に溶解したものに上記（a）で列挙した塩基の弱酸塩（例えば、酢酸塩、グリコール酸塩）を溶解した後に、塩となったポリマーを単離する、（c）上記のカルボキシル基を有する生体内分解性ポリマーを有機溶媒に溶解したものに遷移金属（例，亜鉛，鉄，銅など）の弱酸塩（例えば、酢酸塩、グリコール酸塩）もしくは酸化物を混合した後に塩となったポリマーを単離する、などが挙げられる。

長期間（例えば、６カ月以上）型徐放性製剤用の生体内分解性ポリマーとしては、上記の方法で製造した「末端に遊離のカルボキシル基を有する乳酸－グリコール酸重合体」が好適である。

本発明の組成物における生理活性物質の重量比は、生理活性物質の種類、所望の薬理効果および効果の持続期間などによって異なるが、生理活性物質またはその塩とヒドロキシナフトエ酸またはその塩と生体内分解性ポリマーまたはその塩の三者を含有する徐放性組成物の場合、その三者の和に対して、例えば生理活性ペプチドまたはその塩の場合、約０．００１～約５０重量％、好ましくは約０．０２～約４０重量％、より好ましくは約０．１～３０重量％、最も好ましくは約１４～２４重量％であり、非ペプチド性生理活性物質またはその塩の場合、約０．０１～８０重量％、好ましくは約０．１～５０重量％である。生理活性物質のヒドロキシナフトエ酸塩を含む場合でも同様な重量比である。生理活性ペプチド（仮に（A）と称する）とヒドロキシナフトエ酸（仮に（B）と称する）との塩を含有してなる徐放性組成物の場合、（A）と（B）との塩の和に対して、（A）の重量比は通常約５～約９０重量％、好ましくは約１０～約８５重量％、より好ましくは約１５～約８０重量％、特に好ましくは約３０～約８０重量％である。

生理活性物質またはその塩とヒドロキシナフトエ酸またはその塩と生体内分解性ポリマーまたはその塩の三者を含有する徐放性組成物の場合、ヒドロキシナフトエ酸またはその塩の配合量は、好ましくは、生理活性物質またはその塩１モルに対して、ヒドロキシナフトエ酸またはその塩が約１／２～約２モル、約３／４～約４／３モル、特に好ましくは約４／５～約６／５モルである。

本発明の組成物の設計を、生理活性物質、ヒドロキシナフトエ酸および生体内分解性ポリマーの三者を含有する徐放性組成物について、生理活性物質が塩基性である場合を例に用いて以下に述べる。この場合、組成物中には塩基として生理活性物質が、酸としてヒドロキシナフトエ酸が共存しており、それらが遊離体あるいは塩として組成物中に配合された場合のいずれにおいても、組成物製造時のある時点において含水状態あるいは微量の水の存在下でおのおの解離平衡が成り立っている。微水溶性のヒドロキシナフトエ酸が生理活性物質と形成する塩は、該生理活性物質の特性にもよるが微水溶性と考えられるため、解離平衡はこのような微水溶性塩形成の側に傾く。

塩基性の生理活性物質を高含量に含む組成物を製造するには、上記解離平衡から考えて、生理活性物質のほとんどをプロトン化して上記微水溶性塩にすることが望ましい。このためには、少なくとも生理活性物質またはその塩と当量に近いヒドロキシナフトエ酸またはその塩を配合するのが望ましい。

次に、組成物中に包含された生理活性物質の徐放機構を以下に述べる。生理活性物質は上記の配合組成ではほとんどがプロトン化されて、対イオンを伴った状態で存在している。対イオンは、主にヒドロキシナフトエ酸（好ましくはヒドロキシナフトエ酸）である。組成物が生体中に投与された後は、生体内分解性ポリマーの分解によって経時的にそのオリゴマーおよびモノマーが生成し始めるが、該ポリマーが乳酸－グリコール酸重合体である場合は、生成するオリゴマー（乳酸－グリコール酸オリゴマー）およびモノマー（乳酸またはグリコール酸）は必ず１個のカルボキシル基を有しており、これらも生理活性物質

の対イオンになり得る。生理活性物質の放出は電荷の移動を伴わない、すなわち対イオンを伴った塩として行われるが、移動可能な対イオン種としては上述のようにヒドロキシナフトエ酸、乳酸－グリコール酸オリゴマー（移動可能な程度の分子量の）およびモノマー（乳酸またはグリコール酸）があげられる。

複数の酸が共存する場合には、その組成比にもよるが一般的に強酸の塩が優先的に生ずる。ヒドロキシナフトエ酸のｐＫａは、例えば、３－ヒドロキシ－２－ナフトエ酸のそれは２．７０８（化学便覧　基礎編Ⅱ、日本化学会、昭和４４年９月２５日発行）である。一方、乳酸－グリコール酸オリゴマーのカルボキシル基のそれは知られていないが、乳酸またはグリコール酸のｐＫａ(=3.86 または 3.83)を基礎に、「置換基導入による自由エネルギー変化は加成則で近似可能」との原理に従って計算できる。解離定数に対する置換基の寄与は求められており利用することができる（Table 4.1 in "pKa Prediction for Organic Acid and Bases", D.D.Perrin, B.Dempsey and E.P.Serjeant, 1981）。ヒドロキシル基とエステル結合に対してはそれぞれ、

ΔｐＫａ（ＯＨ）＝－０．９０

ΔｐＫａ（エステル結合）＝－１．７

なので、乳酸－グリコール酸オリゴマーのカルボキシル基のｐＫａは、解離基に最も近いエステル結合の寄与を考慮して、

ｐＫａ ＝ ｐＫａ(乳酸またはグリコール酸)－ΔｐＫａ(OH)＋ΔｐＫａ(エステル結合）＝３．０６または３．０３

と求められる。従って、ヒドロキシナフトエ酸は乳酸（ｐＫａ＝３．８６）、グリコール酸（ｐＫａ＝３．８３）、さらには乳酸－グリコール酸オリゴマーよりも強い酸であるから、上記組成物中ではヒドロキシナフトエ酸と生理活性物質との塩が優先的に生成していると考えられ、その塩の特性が、組成物中からの生理活性物質の徐放特性を支配的に決定すると考えられる。該生理活性物質としては上述の生理活性物質などがあげられる。

ここにおいて、ヒドロキシナフトエ酸が生理活性物質と形成する塩が微水溶性であって水不溶性でないことが徐放機構に好影響をあたえる。すなわち、上記酸解離定数の考察で明らかにしたように移動可能な生理活性物質の塩としては、放出の初期には上記乳酸－グリコール酸オリゴマーおよびモノマーよりも強酸であるヒドロキシナフトエ酸の塩が優勢に存在する結果、その塩の溶解性、体組織への分配性が、生理活性物質の放出速度の決定因子となるため、ヒドロキシナフトエ酸の配合量で薬物の初期放出パターンを調節し得る。その後、ヒドロキシナフトエ酸の減少および生体内分解性ポリマーの加水分解によって生ずるオリゴマーおよびモノマーの増大に伴い、オリゴマーおよびモノマーを対イオンとする生理活性物質の放出機構が徐々に優勢となり、ヒドロキシナフトエ酸が事実上該「組成物」から消失した場合でも安定な生理活性物質の放出が保たれる。また、徐放性組成物の製造時の生理活性物質の取り込み効率をあげること、および取り込まれた生理活性物質の投与後の初期過剰放出を抑制しうることも説明できる。

生理活性ペプチドのヒドロキシナフトエ酸塩を含む徐放性組成物におけるヒドロキシナフトエ酸の役割も前記の機構により説明可能である。

本明細書における「水不溶性」とは、該物質を４０℃以下の温度で、蒸留水中で４時間攪拌したときに、その溶液１Ｌ中に溶解する物質の質量が２５ｍｇ以下の場合をいう。

本明細書における「微水溶性」とは、上記質量が２５ｍｇより大きく、５ｇ以下の場合をいう。該物質が生理活性物質の塩である場合は、上記操作において溶解する生理活性物質の質量をもって上記定義を適用する。

本明細書における徐放性組成物の形態は特に限定されないが、微粒子の形態が好ましく、マイクロスフェア（生体内分解性ポリマーを含む徐放性組成物の場合はマイクロカプセルとも称する）の形態が特に好ましい。また、本明細書におけるマイクロスフェアとは、溶液に分散させることができる注射可能な球

状の微粒子のことをいう。その形態の確認は、例えば、走査型電子顕微鏡による観察で行うことができる。

発明を実施するための最良の形態

本発明の生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩を含有する徐放性組成物、例えば、マイクロカプセルの製造法を例示する。

（Ｉ）水中乾燥法

（ｉ）Ｏ／Ｗ法

本方法においては、まずヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩の有機溶媒溶液を作製する。本発明の徐放性製剤の製造の際に使用する有機溶媒は、沸点が１２０℃以下であることが好ましい。

該有機溶媒としては、例えば、ハロゲン化炭化水素（例、ジクロロメタン、クロロホルム、ジクロロエタン、トリクロロエタン、四塩化炭素等）、エーテル類（例、エチルエーテル、イソプロピルエーテル等）、脂肪酸エステル(例、酢酸エチル、酢酸ブチル等）、芳香族炭化水素（例、ベンゼン、トルエン、キシレン等）、アルコール類（例えば、エタノール、メタノール等）、アセトニトリルなどが用いられる。生体内分解性ポリマーまたはその塩の有機溶媒としてはなかでもジクロロメタンが好ましい。　ヒドロキシナフトエ酸またはその塩の有機溶媒としてはアルコール類が好ましい。　それぞれ別個に溶解した後に混合してもよいし、これらは適宜の割合で混合された有機溶媒中に２者を溶解して用いてもよい。なかでも、ハロゲン化炭化水素とアルコール類との混液が好ましく、特にジクロロメタンとエタノールとの混液が好適である。

ジクロロメタンとの混有機溶媒としてエタノールを用いた場合におけるジクロロメタンとエタノールとの混有機溶媒中のエタノールの含有率は，一般的には約０．０１～約５０％(v/v)、より好ましくは約０．０５～約４０％(v/v)、特

に好ましくは約０．１～約３０％(v/v)から選ばれる。

生体内分解性ポリマーの有機溶媒溶液中の濃度は、生体内分解性ポリマーの分子量、有機溶媒の種類によって異なるが、例えば、ジクロロメタンを有機溶媒として用いた場合、一般的には約０．５～約７０重量％ 、より好ましくは約１～約６０重量％ 、特に好ましくは約２～約５０重量％から選ばれる。

ヒドロキシナフトエ酸またはその塩の有機溶媒中の濃度は、例えばジクロロメタンとエタノールの混液を有機溶媒として用いた場合、一般的には約０．０１～約１０重量％、より好ましくは約０．１～約５重量％、特に好ましくは約０．５～約３重量％から選ばれる。

このようにして得られたヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーの有機溶媒溶液中に、生理活性物質またはその塩を添加し、溶解あるいは分散させる。次いで，得られた生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩から成る組成物を含む有機溶媒溶液を水相中に加え、Ｏ（油相）／Ｗ（水相）エマルションを形成させた後、油相中の溶媒を揮散ないしは水相中に拡散させ、マイクロカプセルを調製する。この際の水相体積は、一般的には油相体積の約１倍～約１０，０００倍、より好ましくは約５倍～約５０，０００倍、特に好ましくは約１０倍～約２，０００倍から選ばれる。

上記の外水相中には乳化剤を加えてもよい。該乳化剤は、一般に安定なＯ／Ｗエマルションを形成できるものであればいずれでもよい。具体的には、例えば、アニオン性界面活性剤（オレイン酸ナトリウム、ステアリン酸ナトリウム、ラウリル硫酸ナトリウムなど）、非イオン性界面活性剤（ポリオキシエチレンソルビタン脂肪酸エステル〔ツイーン(Tween)80、ツイーン(Tween)60、アトラスパウダー社〕、ポリオキシエチレンヒマシ油誘導体〔HCO－60、HCO－50、日光ケミカルズ〕など）、ポリビニルピロリドン、ポリビニルアルコール、カルボキシメチルセルロース、レシチン、ゼラチン、ヒアルロン酸などが用いられ

る。これらの中の１種類か、いくつかを組み合わせて使用してもよい。使用の際の濃度は、好ましくは約０．０１～１０重量％の範囲で、さらに好ましくは約０．０５～約５重量％の範囲で用いられる。

上記の外水相中には浸透圧調節剤を加えてもよい。該浸透圧調節剤としては、水溶液とした場合に浸透圧を示すものであればよい。

該浸透圧調節剤としては、例えば、多価アルコール類、一価アルコール類、単糖類、二糖類、オリゴ糖およびアミノ酸類またはそれらの誘導体などが挙げられる。

上記の多価アルコール類としては、例えば、グリセリン等の三価アルコール類、アラビトール，キシリトール，アドニトール等の五価アルコール類、マンニトール，ソルビトール，ズルシトール等の六価アルコール類などが用いられる。なかでも、六価アルコール類が好ましく、特にマンニトールが好適である。

上記の一価アルコール類としては、例えば、メタノール、エタノール、イソプロピルアルコールなどが挙げられ、このうちエタノールが好ましい。

上記の単糖類としては、例えば、アラビノース，キシロース，リボース，２－デオキシリボース等の五炭糖類、ブドウ糖，果糖，ガラクトース，マンオース，ソルボース，ラムノース，フコース等の六炭糖類が用いられ、このうち六炭糖類が好ましい。

上記のオリゴ糖としては、例えば、マルトトリオース，ラフィノース糖等の三糖類、スタキオース等の四糖類などが用いられ、このうち三糖類が好ましい。

上記の単糖類、二糖類およびオリゴ糖の誘導体としては、例えば、グルコサミン、ガラクトサミン、グルクロン酸、ガラクツロン酸などが用いられる。

上記のアミノ酸類としては、Ｌ－体のものであればいずれも用いることができ、例えば、グリシン、ロイシン、アルギニンなどが挙げられる。このうちＬ－アルギニンが好ましい。

これらの浸透圧調節剤は単独で使用しても、混合して使用してもよい。

これらの浸透圧調節剤は、外水相の浸透圧が生理食塩水の浸透圧の約１／５０～約５倍、好ましくは約１／２５～約３倍となる濃度で用いられる。

有機溶媒を除去する方法としては、自体公知の方法あるいはそれに準じる方法が用いられる。例えば、プロペラ型撹拌機またはマグネチックスターラーや超音波発生装置などで撹拌しながら常圧もしくは徐々に減圧にして有機溶媒を蒸発させる方法、ロータリーエヴァポレーターなどを用いて真空度を調節しながら有機溶媒を蒸発させる方法、透析膜を用いて徐々に有機溶媒を除去する方法などが挙げられる。

このようにして得られたマイクロカプセルは遠心分離または濾過して分取した後、マイクロカプセルの表面に付着している遊離の生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩、薬物保持物質、乳化剤などを蒸留水で数回繰り返し洗浄し、再び蒸留水などに分散して凍結乾燥する。

製造工程中、粒子同士の凝集を防ぐために凝集防止剤を加えてもよい。該凝集防止剤としては、例えば、マンニトール，ラクトース，ブドウ糖，デンプン類（例、コーンスターチ等）などの水溶性多糖、グリシンなどのアミノ酸、フィブリン，コラーゲンなどのタンパク質などが用いられる。なかでも、マンニトールが好適である。

また、凍結乾燥後、必要であれば、減圧下マイクロカプセルが同士が融着しない条件内で加温してマイクロカプセル中の水分および有機溶媒の除去を行ってもよい。好ましくは、毎分１０～２０℃の昇温速度の条件下で示差走査熱量計で求めた生体内分解性ポリマーの中間点ガラス転移温度よりも若干高い温度で加温する。より好ましくは生体内分解性ポリマーの中間点ガラス転移温度からこれより約３０℃高い温度範囲内で加温する。とりわけ，生体内分解性ポリマーとして乳酸－グリコール酸重合体を用いる場合には好ましくはその中間点ガラス転移温度以上中間点ガラス転移温度より１０℃高い温度範囲，さらに好ましくは、中間点ガラス転移温度以上中間点ガラス転移温度より５℃高い温度

範囲で加温する。

加温時間はマイクロカプセルの量などによって異なるものの、一般的にはマイクロカプセル自体が所定の温度に達した後、約１２時間～約１６８時間、好ましくは約２４時間～約１２０時間、特に好ましくは約４８時間～約９６時間である。

加温方法は、マイクロカプセルの集合が均一に加温できる方法であれば特に限定されない。

該加温乾燥方法としては、例えば、恒温槽、流動槽、移動槽またはキルン中で加温乾燥する方法、マイクロ波で加温乾燥する方法などが用いられる。このなかで恒温槽中で加温乾燥する方法が好ましい。

（ii）W／O／W法（１）

まず、生体内分解性ポリマーまたはその塩の有機溶媒溶液を調製する。

該有機溶媒ならびに生体内分解性ポリマーまたはその塩の有機溶媒溶液中の濃度は、前記（Ｉ）（ｉ）項に記載と同様である。また混有機溶媒を用いる場合には、その両者の比率は、前記（Ｉ）（ｉ）項に記載と同様である。

このようにして得られた生体内分解性ポリマーまたはその塩の有機溶媒溶液中に、生理活性物質またはその塩を添加し、溶解あるいは分散させる。次いで、得られた生理活性物質またはその塩と生体内分解性ポリマーまたはその塩からなる組成物を含む有機溶媒溶液（油相）にヒドロキシナフトエ酸またはその塩の溶液〔該溶媒としては、水、アルコール類（例、メタノール、エタノール等）の水溶液、ピリジン水溶液、ジメチルアセトアミド水溶液等）〕を添加する。この混合物をホモジナイザーまたは超音波等の公知の方法で乳化し、W／Oエマルションを形成させる。

次いで，得られた生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩から成るW／Oエマルションを水相中に加え、W（内水相）/O（油相）/ W（外水相）エマルションを形成

させた後、油相中の溶媒を揮散させ、マイクロカプセルを調製する。この際の外水相体積は一般的には油相体積の約１倍～約１０，０００倍、より好ましくは約５倍～約５，０００倍、特に好ましくは約１０倍～約２，０００倍から選ばれる。

上記の外水相中に加えてもよい乳化剤や浸透圧調節剤、およびその後の調製法は前記（Ｉ）（ｉ）項に記載と同様である。

（iii）Ｗ／Ｏ／Ｗ法（２）

まず、ヒドロキシナフトエ酸またはその塩と生体内分解性ポリマーまたはその塩の有機溶媒溶液を作成し、そうして得られた有機溶媒溶液を油相と称する。該作成法は、前記（Ｉ）（ｉ）項に記載と同様である。あるいは、ヒドロキシナフトエ酸またはその塩と生体内分解性ポリマーをそれぞれ別々に有機溶媒溶液として作成し、その後に混合してもよい。生体内分解性ポリマーの有機溶媒溶液中の濃度は、生体内分解性ポリマーの分子量、有機溶媒の種類によって異なるが、例えば、ジクロロメタンを有機溶媒として用いた場合、一般的には約０．５～約７０重量％、より好ましくは約１～約６０重量％、特に好ましくは約２～約５０重量％から選ばれる。

次に生理活性物質またはその塩の溶液または分散液〔該溶媒としては、水、水とアルコール類（例、メタノール、エタノール等）などとの混液〕を作成する。

生理活性物質またはその塩の添加濃度は一般的には０．００１ｍｇ／ｍｌ～１０ｇ／ｍｌ、より好ましくは０．１ｍｇ／ｍｌ～５ｇ／ｍｌで更に好ましくは１０ｍｇ／ｍｌ～３ｇ／ｍｌである。

溶解補助剤、安定化剤として公知のものを用いてもよい。生理活性物質や添加剤の溶解あるいは分散には活性が失われない程度に加熱、振とう、撹拌などを行ってもよく、そうして得られた水溶液を内水相と称する。

上記により得られた内水相と油相とをホモジナイザーまたは超音波等の公知

の方法で乳化し、W／Oエマルションを形成させる。

混合する油相の体積は内水相の体積に対し、約１～約１０００倍、好ましくは約２～１００倍、より好ましくは約３～１０倍である。

得られたW／Oエマルションの粘度範囲は一般的には約１５～２０℃で、約１０～１０，０００ｃｐで、好ましくは約１００～５，０００ｃｐである。さらに好ましくは約５００～2，０００ｃｐである。

次いで，得られた生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩から成るW／Oエマルションを水相中に加え、W（内水相）/O（油相）/ W（外水相）エマルションを形成させた後、油相中の溶媒を揮散ないしは外水相中に拡散させ、マイクロカプセルを調製する。この際の外水相体積は一般的には油相体積の約１倍～約１０，０００倍、より好ましくは約５倍～約５０，０００倍、特に好ましくは約１０倍～約２，０００倍から選ばれる。

上記の外水相中に加えてもよい乳化剤や浸透圧調節剤、およびその後の調製法は前記（Ｉ）（ｉ）項に記載と同様である。

（II）相分離法

本法によってマイクロカプセルを製造する場合には，前記（Ｉ）の水中乾燥法に記載した生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩の３者から成る組成物を含む有機溶媒溶液にコアセルベーション剤を撹拌下徐々に加えてマイクロカプセルを析出，固化させる。該コアセルベーション剤は油相体積の約０．０１～１，０００倍、好ましくは約０．０５～５００倍、特に好ましくは約０．１～２００倍から選ばれる。

コアセルベーション剤としては、有機溶媒と混和する高分子系，鉱物油系または植物油系の化合物等で生理活性物質またはその塩のヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩の複合体を溶解しない

ものであれば特に限定はされない。具体的には、例えば、シリコン油，ゴマ油，大豆油，コーン油，綿実油，ココナッツ油，アマニ油，鉱物油，n－ヘキサン，n－ヘプタンなどが用いられる。これらは２種類以上混合して使用してもよい。

このようにして得られたマイクロカプセルを分取した後、ヘプタン等で繰り返し洗浄して生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩からなる組成物以外のコアセルベーション剤等を除去し、減圧乾燥する。もしくは、前記（Ｉ）（ｉ）の水中乾燥法で記載と同様の方法で洗浄を行った後に凍結乾燥、さらには加温乾燥する。

（III）噴霧乾燥法

本法によってマイクロカプセルを製造する場合には，前記（Ｉ）の水中乾燥法に記載した生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩の３者を含有する有機溶媒溶液をノズルを用いてスプレードライヤー（噴霧乾燥器）の乾燥室内に噴霧し、極めて短時間内に微粒化液滴内の有機溶媒を揮発させ、マイクロカプセルを調製する。該ノズルとしては、例えば、二流体ノズル型，圧力ノズル型，回転ディスク型等がある。この後、必要であれば、前記（Ｉ）の水中乾燥法で記載と同様の方法で洗浄を行った後に凍結乾燥、さらには加温乾燥してもよい。

上述のマイクロカプセル以外の剤形としてマイクロカプセルの製造法（Ｉ）の水中乾燥法に記載した生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩を含む有機溶媒溶液を例えばロータリーエヴァポレーターなどを用いて真空度を調節しながら有機溶媒および水を蒸発させて乾固した後、ジェットミルなどで粉砕して微粉末（マイクロパーティクルとも称する）としてもよい。

さらには、粉砕した微粉末をマイクロカプセルの製造法（Ｉ）の水中乾燥法で記載と同様の方法で洗浄を行った後に凍結乾燥、さらには加温乾燥してもよい。

ここで得られるマイクロカプセルまたは微粉末は使用する生体内分解性ポリマーまたは乳酸－グリコール酸重合体の分解速度に対応した薬物放出が達成できる。

次に、本発明の生理活性物質のヒドロキシナフトエ酸塩を含む徐放性組成物の製造法について例示する。 本製造法においては生理活性物質として、生理活性ペプチドが好ましく用いられる。

（ＩＶ）２ステップ法

生理活性物質またはその塩を上述の生理活性物質の配合量の定義で示した重量比率になるようにヒドロキシナフトエ酸またはその塩の有機溶媒溶液に加え、生理活性物質のヒドロキシナフトエ酸塩を含有する有機溶媒溶液を作る。 該有機溶媒としては、前記（Ｉ）（ｉ）に記載と同様である。また混有機溶媒を用いる場合には、その両者の比率は、前記（Ｉ）（ｉ）項に記載と同様である。

生理活性物質のヒドロキシナフトエ酸塩を含有する組成物を析出させるための有機溶媒を除去する方法は、自体公知の方法あるいはそれに準じる方法が用いられる。例えば、ロータリーエヴァポレーターなどを用いて真空度を調節しながら有機溶媒を蒸発させる方法などが挙げられる。

このようにして得られた生理活性物質のヒドロキシナフトエ酸塩を含有する組成物の有機溶媒溶液を再度作り、徐放性組成物（マイクロスフェアまたは微粒子）を作製することができる。

該有機溶媒としては、例えば、ハロゲン化炭化水素（例、ジクロロメタン、クロロホルム、ジクロロエタン、トリクロロエタン、四塩化炭素等）、エーテル類（例、エチルエーテル、イソプロピルエーテル等）、脂肪酸エステル(例、酢酸エチル、酢酸ブチル等）、芳香族炭化水素（例、ベンゼン、トルエン、キシレン等）などが用いられる。これらは適宜の割合で混合して用いてもよい。なかでも、ハロゲン化炭化水素が好ましく、特にジクロロメタンが好適である。

次いで，得られた生理活性物質のヒドロキシナフトエ酸塩を含有する組成物

を含む有機溶媒溶液を水相中に加え、O（油相）／W（水相）エマルションを形成させた後、油相中の溶媒を蒸発させ、マイクロスフェアを調製する。この際の水相体積は、一般的には、油相体積の約１倍～約１０，０００倍、より好ましくは約５倍～約５，０００倍、特に好ましくは約１０倍～約２，０００倍から選ばれる。

上記の外水相中に加えてもよい乳化剤や浸透圧調節剤、およびその後の調製法は前記（Ｉ）（ｉ）項に記載と同様である。

有機溶媒を除去する方法としては、自体公知の方法あるいはそれに準じる方法が用いられる。例えば、プロペラ型撹拌機またはマグネチックスターラーなどで撹拌しながら、常圧もしくは徐々に減圧にして有機溶媒を蒸発させる方法、ロータリーエヴァポレーターなどを用いて真空度を調節しながら有機溶媒を蒸発させる方法などが挙げられる。

このようにして得られたマイクロスフェアは遠心分離または濾過して分取した後、マイクロスフェアの表面に付着している遊離の生理活性物質、ヒドロキシナフトエ酸、乳化剤などを蒸留水で数回繰り返し洗浄し、再び蒸留水などに分散して凍結乾燥する。

製造工程中、粒子同士の凝集を防ぐために凝集防止剤を加えてもよい。該凝集防止剤としては、例えば、マンニトール，ラクトース，ブドウ糖，デンプン類（例、コーンスターチ等）などの水溶性多糖、グリシンなどのアミノ酸、フィブリン，コラーゲンなどのタンパク質などが挙げられる。なかでも、マンニトールが好ましい。

また、凍結乾燥後、必要であれば、減圧下マイクロスフェアが同士が融着しない条件内で加温してマイクロスフェア中の水分および有機溶媒の除去をさらに行ってもよい。

加温時間はマイクロスフェアの量などによって異なるものの、一般的にはマイクロスフェア自体が所定の温度に達した後、約１２時間～約１６８時間、好

ましくは約２４時間～約１２０時間、特に好ましくは約４８時間～約９６時間である。

加温方法は、マイクロスフェアの集合が均一に加温できる方法であれば特に限定されない。

該加温乾燥方法としては、例えば、恒温槽、流動槽、移動槽またはキルン中で加温乾燥する方法、マイクロ波で加温乾燥する方法などが用いられる。このなかで恒温槽中で加温乾燥する方法が好ましい。得られたマイクロスフェアは比較的均一な球状の形態をしており、注射投与時の抵抗が少なく、針つまりを起こしにくい。また、細い注射針を使うことができるため、注射時の患者の苦痛が軽減される。

（Ｖ）１ステップ法

生理活性物質またはその塩を上述の生理活性物質の配合量の定義で示した重量比率になるようにヒドロキシナフトエ酸またはその塩の有機溶媒溶液に加え、生理活性物質のヒドロキシナフトエ酸塩を含有する有機溶媒溶液を作り、徐放性製剤（マイクロスフェアまたは微粒子）を作製する。

該有機溶媒としては、前記（Ｉ）（ｉ）に記載と同様である。また混有機溶媒を用いる場合には、その両者の比率は、前記（Ｉ）（ｉ）項に記載と同様である。

次いで，生理活性物質のヒドロキシナフトエ酸塩を含有する有機溶媒溶液を水相中に加え、Ｏ（油相）／Ｗ（水相）エマルションを形成させた後、油相中の溶媒を蒸発させ、マイクロスフェアを調製する。この際の水相体積は、一般的には油相体積の約１倍～約１０，０００倍、より好ましくは約５倍～約５，０００倍、特に好ましくは約１０倍～約２，０００倍から選ばれる。

上記の外水相中に加えてもよい乳化剤や浸透圧調節剤、およびその後の調製法は前記(Ｉ Ｖ)項に記載と同様である。

本発明の徐放性組成物は、マイクロスフェア、マイクロカプセル、微粉末（マ

イクロパーティクル）など何れの形態であってもよいが、生理活性物質とヒドロキシナフトエ酸との２者から成る場合はマイクロスフェアが、生理活性物質とヒドロキシナフトエ酸と生体内分解性ポリマーとの３者から成る場合はマイクロカプセルが好適である。

本発明の徐放性組成物は、そのままままたはこれらを原料物質として種々の剤形に製剤化し、筋肉内、皮下、臓器などへの注射剤または埋め込み剤、鼻腔、直腸、子宮などへの経粘膜剤、経口剤（例、カプセル剤（例、硬カプセル剤、軟カプセル剤等）、顆粒剤、散剤等の固形製剤、シロップ剤、乳剤、懸濁剤等の液剤等）などとして投与することができる。

例えば、本発明の徐放性組成物を注射剤とするには、これらを分散剤（例、ツイーン（Tween）80，HCO－60等の界面活性剤、ヒアルロン酸ナトリウム，カルボキシメチルセルロース，アルギン酸ナトリウム等の多糖類など）、保存剤（例、メチルパラベン、プロピルパラベンなど）、等張化剤（例、塩化ナトリウム，マンニトール，ソルビトール，ブドウ糖，プロリンなど）等と共に水性懸濁剤とするか、ゴマ油、コーン油などの植物油と共に分散して油性懸濁剤として実際に使用できる徐放性注射剤とすることができる。

本発明の徐放性組成物の粒子径は、懸濁注射剤として使用する場合には、その分散度、通針性を満足する範囲であればよく、例えば、平均粒子径として約０．１～３００μm、好ましくは約０．５～１５０μmの範囲、さらに好ましくは約１から１００μmの範囲である。

本発明の徐放性組成物を無菌製剤にするには、製造全工程を無菌にする方法、ガンマ線で滅菌する方法、防腐剤を添加する方法等が挙げられるが、特に限定されない。

本発明の徐放性組成物は、低毒性であるので、哺乳動物（例、ヒト、牛、豚、犬、ネコ、マウス、ラット、ウサギ等）に対して安全な医薬などとして用いることができる。

本発明の徐放性組成物の投与量は、主薬である生理活性物質の種類と含量、剤形、生理活性物質放出の持続時間、対象疾病、対象動物などによって種々異なるが、生理活性物質の有効量であればよい。主薬である生理活性物質の１回当たりの投与量としては、例えば、徐放性製剤が６カ月製剤である場合、好ましくは、成人１人当たり約０．０１ｍｇ～１０ｍｇ／ｋｇ体重の範囲，さらに好ましくは約０．０５ｍｇ～５ｍｇ／ｋｇ体重の範囲から適宜選ぶことができる。

１回当たりの徐放性組成物の投与量は、成人１人当たり好ましくは、約０．０５ｍｇ～５０ｍｇ／ｋｇ体重の範囲、さらに好ましくは約０．１ｍｇ～３０ｍｇ／ｋｇ体重の範囲から適宜選ぶことができる。

投与回数は、数週間に１回、１か月に１回、または数か月（例、３ヵ月、４ヵ月、６ヵ月など）に１回等、主薬である生理活性物質の種類と含量、剤形、生理活性物質放出の持続時間、対象疾病、対象動物などによって適宜選ぶことができる。

本発明の徐放性組成物は、含有する生理活性物質の種類に応じて、種々の疾患などの予防・治療剤として用いることができるが、例えば、生理活性物質が、ＬＨ－ＲＨ誘導体である場合には、ホルモン依存性疾患、特に性ホルモン依存性癌（例、前立腺癌、子宮癌、乳癌、下垂体腫瘍など）、前立腺肥大症、子宮内膜症、子宮筋腫、思春期早発症、月経困難症、無月経症、月経前症候群、多房性卵巣症候群等の性ホルモン依存性の疾患の予防・治療剤、および避妊（もしくは、その休薬後のリバウンド効果を利用した場合には、不妊症の予防・治療）剤などとして用いることができる。さらに、性ホルモン非依存性であるがＬＨ－ＲＨ感受性である良性または悪性腫瘍などの予防・治療剤としても用いることができる。

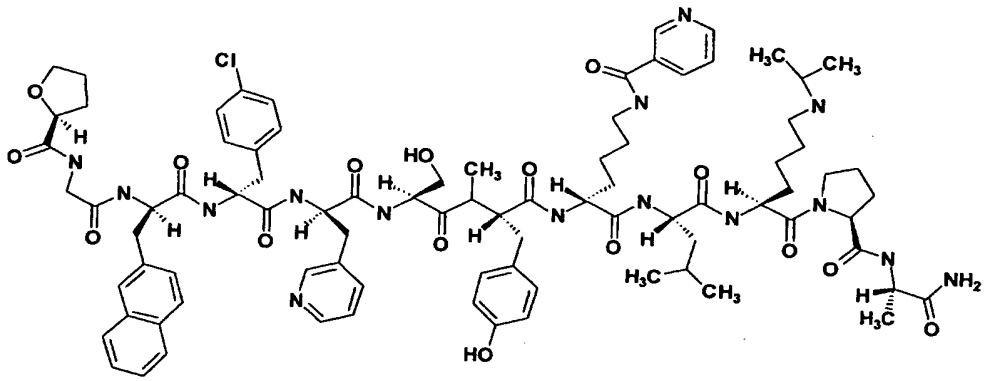
以下に実施例、実験例および比較例をあげて本発明をさらに具体的に説明す

るが、これらは本発明を限定するものではない。

実施例１

N-(S)-Tetrahydrofur-2-oyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH$_2$ （以下ペプチドＡと略記する）の酢酸塩（ＴＡＰ社製）3429.6m および３－ヒドロキシ－２－ナフトエ酸（和光純薬工業製）685.2mg をエタノール 15ml に溶解した。

（ペプチドＡの構造式）



この溶液をロータリーエヴァポレーターを用いて徐々に減圧にし、有機溶媒を蒸発させた。この残留物をジクロロメタン 5.5ml に再溶解し、予め 18℃に調節しておいた 0.1%(w/w)ポリビニルアルコール（EG－40、日本合成化学製）水溶液 400ml 中に注入し、タービン型ホモミキサーを用いて 8,000rpm で攪拌しＯ／Ｗエマルションとした。このＯ／Ｗエマルションを室温で３時間撹拌してジクロロメタンを揮散させ、油相を固化させた後、７５μｍの目開きの篩いを用いて篩過し、遠心分離機（05PR－22、日立製作所）を用いて 2,000rpm、5 分間の条件でマイクロスフェアを沈降させて捕集した。これを再び蒸留水に分散後、さらに遠心分離を行い、遊離薬物等を洗浄し、マイクロスフェアを捕集した。捕集されたマイクロスフェアは少量の蒸留水を加えて再分散後、凍結乾燥して

粉末として得られた。マイクロスフェアの質量回収率は６５％で、マイクロスフェア中のペプチドＡ含量および３－ヒドロキシ－２－ナフトエ酸／ペプチドＡモル比はそれぞれ７５．４％、１．９４であった。

実施例２

ペプチドＡの酢酸塩 1785.1mg および３－ヒドロキシ－２－ナフトエ酸 1370.4mg をエタノール 15ml に溶解した。この溶液をロータリーエヴァポレーターを用いて徐々に減圧にし、有機溶媒を蒸発させた。この残留物をジクロロメタン 10ml に再溶解し、予め 18℃に調節しておいた 0.1%(w/w)ポリビニルアルコール水溶液 1000ml 中に注入し、タービン型ホモミキサーを用いて 8,000rpm で撹拌しＯ／Ｗエマルションとした。その後の操作は実施例１に記載と同様にしてマイクロスフェアを得た。マイクロスフェアの質量回収率は５８％で、マイクロスフェア中のペプチドＡ含量および３－ヒドロキシ－２－ナフトエ酸／ペプチドＡモル比はそれぞれ５４．３％、６．１５であった。

実施例３

ペプチドＡの酢酸塩 1800mg、３－ヒドロキシ－２－ナフトエ酸 173mg および乳酸－グリコール酸共重合体（乳酸／グリコール酸=50/50（モル%）、重量平均分子量 10,100、数平均分子量 5,670、アルカリ滴定によるカルボキシル基量 ２６８．８μ mol/g、和光純薬工業製）2g をジクロロメタン 6ml およびエタノール 0.2ml の混有機溶媒に溶解し、予め 18℃に調節しておいた 5%マンニトール含有 0.1%(w/w)ポリビニルアルコール水溶液 900ml 中に注入し，タービン型ホモミキサーを用いて 7,000rpm で撹拌しＯ／Ｗエマルションとした。このＯ／Ｗエマルションを室温で３時間撹拌してジクロロメタンおよびエタノールを揮散あるいは水相中に拡散させ、油相を固化させた後、７５μｍの目開きの篩いを用いて篩過し、遠心分離機を用いて 2,000rpm、5 分間の条件でマイクロカプセル

を沈降させて捕集した。これを再び蒸留水に分散後、さらに遠心分離を行い、遊離薬物等を洗浄し、マイクロカプセルを捕集した。捕集されたマイクロカプセルは 250mg のマンニトールと少量の蒸留水を加えて再分散後、凍結乾燥して粉末として得られた。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は７６％、マイクロカプセル中のペプチドＡ含量および３－ヒドロキシー２－ナフトエ酸／ペプチドＡモル比はそれぞれ３４．７％、１．１９であった。そしてこの実現含量を仕込み含量で除して求めた封入効率は、８４．６％であった。

実施例４

ペプチドＡの酢酸塩 1900mg、３－ヒドロキシー２－ナフトエ酸 182mg および乳酸－グリコール酸共重合体（実施例３に同じ）1.9g をジクロロメタン 6ml およびエタノール 0.2ml の混有機溶媒に溶解し、予め 18℃に調節しておいた 5% マンニトールと 0.05% L－アルギニン含有 0.1%(w/w)ポリビニルアルコール水溶液 900ml 中に注入し，タービン型ホモミキサーを用い、7,000rpm で撹拌してO／Wエマルションとした。その後の操作は実施例３に記載と同様にしてマイクロカプセルを得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は８５％、マイクロカプセル中のペプチドＡ含量および３－ヒドロキシー２－ナフトエ酸／ペプチドＡモル比はそれぞれ３８．６％、０．８３であった。そしてこの実現含量を仕込み含量で除して求めた封入効率は、８８．９％であった。

実施例５

実施例４に記載の乳酸－グリコール酸共重合体を乳酸／グリコール酸 =75/25（モル%）、重量平均分子量 10,700、数平均分子量 6,100、アルカリ滴定によるカルボキシル基量　２６５．３μ mol/g の乳酸－グリコール酸共重合体

に変更し、ジクロロメタン量を 6.5ml に変更した以外は、実施例４に記載と同様にしてマイクロカプセルを得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は８７％、マイクロカプセル中のペプチドＡ含量および３－ヒドロキシー２－ナフトエ酸／ペプチドＡモル比はそれぞれ３８．３％、０．９２であった。そしてこの実現含量を仕込み含量で除して求めた封入効率は、８８．３％であった。

実施例６

ペプチドＡの酢酸塩 1800mg および乳酸－グリコール酸共重合体（乳酸／グリコール酸=50/50（モル%）、重量平均分子量 12,700、数平均分子量 7,090、アルカリ滴定によるカルボキシル基量　２０９．２μ mol/g、和光純薬工業製）1.8g をジクロロメタン 7.2ml に溶解した溶液に、３－ヒドロキシー２－ナフトエ酸ナトリウム塩 196mg を水 2.3ml に溶解した溶液を加えホモジナイザーで乳化しW／Ｏエマルションを調製した。このエマルションを予め 18℃に調節しておいた 5%マンニトール含有 0.1%(w/w)ポリビニルアルコール水溶液 800ml 中に注入し，タービン型ホモミキサーを用いて 7,000rpm で撹拌しW／Ｏ／Wエマルションとした。このW／Ｏ／Wエマルションを室温で３時間撹拌してジクロロメタンおよびエタノールを揮散あるいは水相中に拡散させ、油相を固化させた後、７５μmの目開きの篩いを用いて篩過し、遠心分離機を用いて 2,000rpm、5 分間の条件でマイクロカプセルを沈降させて捕集した。これを再び蒸留水に分散後、さらに遠心分離を行い、遊離薬物等を洗浄し、マイクロカプセルを捕集した。捕集されたマイクロカプセルは 250mg のマンニトールと少量の蒸留水を加えて再分散後、凍結乾燥して粉末として得られた。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は７９％、マイクロカプセル中のペプチドＡ含量および３－ヒドロキシー２－ナフトエ酸／ペプチドＡモル比はそれぞれ３２．８％、０．９１であった。そしてこの実現含量を仕込み

含量で除して求めた封入効率は、８１．２％であった。

実験例１

実施例１、２で得られた各マイクロスフェア約４０ｍｇ、または実施例３～５で得られた各マイクロカプセル約６０ｍｇを０．５ｍｌの分散媒（０．２５ｍｇのカルボキシメチルセルロース，０．５ｍｇのポリソルベート８０，２５ｍｇのマンニトールを溶解した蒸留水）に分散して８～１０週齢雄性ＳＤラットの背部皮下に２２Ｇ注射針で投与した。投与から所定時間後にラットを屠殺して投与部位に残存するマイクロスフェアまたはマイクロカプセルを取り出し、この中のペプチドＡを定量してそれぞれの初期含量で除して求めた残存率を表１に示す。

表１

	１日	１週	２週	３週	４週
実施例１	７３％	３０％	１１％	６％	６％
実施例２	８５％	３７％	９％	１％	
実施例３	７０％	３１％	１４％	９％	
実施例４	７７％	２９％	１１％	１０％	６％
実施例５	８１％	４４％	２５％	１７％	１３％

実施例１および２の実験結果より、ペプチドＡと３－ヒドロキシ－２－ナフトエ酸の２者からなるマイクロスフェアからのペプチドＡの放出は、両者の比率の違いにより異なり、３－ヒドロキシ－２－ナフトエ酸の割合が多いほうがペプチドＡの放出が速やかであった。また、実施例３、４および５の実験結果より、乳酸－グリコール酸共重合体を加えた３者からなるマイクロカプセルでは、２者のみからなるマイクロスフェアからのペプチドＡの放出性とは異なる結果

が得られ、さらには乳酸－グリコール酸共重合体の組成、重量平均分子量および末端カルボキシル基量の異なるものを組み合わせることによりその放出挙動を制御できることが明らかとなった。

実施例７

5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5（以下、ペプチドＢと略記する。武田薬品製）の酢酸塩 0.8g を 0.8ml の蒸留水に溶解した溶液を、DL-乳酸重合体（重量平均分子量 36,000、数平均分子量 18,000、ラベル化定量法によるカルボキシル基量 70.4 μ mol/g）3.08g および３－ヒドロキシ－２－ナフトエ酸 0.12g をジクロロメタン 5ml およびエタノール 0.3ml の混有機溶媒で溶解した溶液と混合してホモジナイザーで乳化し、Ｗ／Ｏエマルションを形成した。次いでこのＷ／Ｏエマルションを、予め 15℃に調節しておいた 0.1% (w/w) ポリビニルアルコール（EG-40、日本合成化学製）水溶液 800ml 中に注入し、タービン型ホモミキサーを用いて 7,000rpm で撹拌しＷ／Ｏ／Ｗエマルションとした。このＷ／Ｏ／Ｗエマルションを室温で 3 時間撹拌してジクロロメタンおよびエタノールを揮散ないしは外水相中に拡散させ、油相を固化させた後、75 μ m の目開きの篩いを用いて篩過し、次いで遠心分離機（05PR-22、日立製作所）を用いて 2,000rpm、5 分間の条件でマイクロカプセルを沈降させて捕集した。これを再び蒸留水に分散後、さらに遠心分離を行い、遊離薬物等を洗浄し、マイクロカプセルを捕集した。捕集されたマイクロカプセルは少量の蒸留水を加えて再分散後、凍結乾燥して粉末として得られた。マイクロカプセルの質量回収率は 46%、マイクロカプセル中のペプチドＢ含量は 21.3%、３－ヒドロキシ－２－ナフトエ酸含量は 2.96%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドＢにおいて 106.6%、３－ヒドロキシ－２－ナフトエ酸において 98.6%であった。

実施例８

ペプチドＢの酢酸塩 1.2g を 1.2ml の蒸留水に溶解した溶液を、DL-乳酸重合体（重量平均分子量 25,200、数平均分子量 12,800、ラベル化定量法によるカルボキシル基量 62.5 μ mol/g）4.62g および３－ヒドロキシ－２－ナフトエ酸 0.18g をジクロロメタン 7.5ml およびエタノール 0.45ml の混有機溶媒で溶解した溶液と混合してホモジナイザーで乳化し、Ｗ／Ｏエマルションを形成した。次いでこのＷ／Ｏエマルションを、予め 15℃に調節しておいた 0.1% (w/w)ポリビニルアルコール（EG-40、日本合成化学製）水溶液 1200ml 中に注入し、タービン型ホモミキサーを用いて 7,000rpm で撹拌しＷ／Ｏ／Ｗエマルションとした。このＷ／Ｏ／Ｗエマルションを室温で 3 時間撹拌してジクロロメタンおよびエタノールを揮散ないしは外水相中に拡散させ、油相を固化させた後、75 μ m の目開きの篩いを用いて篩過し、次いで遠心分離機（05PR-22、日立製作所）を用いて 2,000rpm、5 分間の条件でマイクロカプセルを沈降させて捕集した。これを再び蒸留水に分散後、さらに遠心分離を行い、遊離薬物等を洗浄し、マイクロカプセルを捕集した。捕集されたマイクロカプセルは少量の蒸留水に再分散し、マンニトール 0.3g を添加して溶解した後凍結乾燥して粉末として得られた。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は 55.2%、マイクロカプセル中のペプチドＢ含量は 21.3%、３－ヒドロキシ－２－ナフトエ酸含量は 2.96%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドＢにおいて 99.7%、３－ヒドロキシ－２－ナフトエ酸において 102.2%であった。

実施例９

実施例８に記載のＤＬ－乳酸重合体を、ＤＬ－乳酸重合体（重量平均分子量 28,800、数平均分子量 14,500、ラベル化定量法によるカルボキシル基量 78.1 μ mol/g）とした以外は実施例８に記載と同様にしてマイクロカプセル粉末を

得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は５０．２%、マイクロカプセル中のペプチドＢ含量は２０．８%、３－ヒドロキシー２－ナフトエ酸含量は２．７８%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドＢにおいて１０３．４ %、３－ヒドロキシー２－ナフトエ酸において９２．７%であった。

比較例１

ペプチドＢの酢酸塩 1.2g を 1.2ml の蒸留水に溶解した溶液を、実施例９と同じ DL-乳酸重合体 4.8g をジクロロメタン 7.8ml で溶解した溶液と混合してホモジナイザーで乳化し、 W／Oエマルションを形成した。次いでこのW／Oエマルションを、予め 15℃に調節しておいた 0.1% (w/w)ポリビニルアルコール（EG-40、日本合成化学製）水溶液 1200ml 中に注入し、タービン型ホモミキサーを用い、7,000rpm でW／O／Wエマルションとした。以下実施例８と同様に操作してマイクロカプセル粉末を得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は 53.6%、マイクロカプセル中のペプチドＢ含量は 12.1%、であった。そしてこの実現含量を仕込み含量で除して求めたペプチドＢの封入率は 60.6%であって、実施例９に比べてはるかに低い。従って３－ヒドロキシ２－ナフトエ酸の添加によりペプチドＢの封入効率が上昇したことは明らかである。

実施例１０

ペプチドＢの酢酸塩 1.00g を 1.00ml の蒸留水に溶解した溶液を、DL-乳酸重合体（重量平均分子量 49,500、数平均分子量 17,500、ラベル化定量法によるカルボキシル基量 45.9 μ mol/g） 3.85g および３－ヒドロキシー２－ナフトエ酸 0.15g をジクロロメタン 7.5ml およびエタノール 0.4ml の混有機溶媒で溶解した溶液と混合してホモジナイザーで乳化し、 W／Oエマルションを形成した。

以下0.1%(w/w)ポリビニルアルコール水溶液の液量を1000ml、マンニトールの添加量を 0.257g とした以外は実施例８に記載と同様にしてマイクロカプセル粉末を得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は53.8%、マイクロカプセル中のペプチドＢ含量は18.02%、３－ヒドロキシー２－ナフトエ酸含量は 2.70%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドＢにおいて90.1 %、３－ヒドロキシー２－ナフトエ酸において90.1%であった。

実施例１１

ペプチドＢの酢酸塩1.202g を1.20ml の蒸留水に溶解した溶液を、DL-乳酸重合体（重量平均分子量19,900、数平均分子量10,700、ラベル化定量法によるカルボキシル基量104.6 μ mol/g）4.619 および３－ヒドロキシー２－ナフトエ酸0.179g をジクロロメタン 7.5ml およびエタノール 0.45ml の混有機溶媒で溶解した溶液と混合してホモジナイザーで乳化し、Ｗ／Ｏエマルションを形成した。以下マンニトールの添加量を 0.303g とした以外は実施例８に記載と同様にしてマイクロカプセル粉末を得た。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は 61.4%、マイクロカプセル中のペプチドＢ含量は15.88%、３－ヒドロキシー２－ナフトエ酸含量は2.23%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドＢにおいて77.75%、３－ヒドロキシー２－ナフトエ酸において75.05%であった。

実施例１２

ペプチドＢの酢酸塩1.00g を1.00ml の蒸留水に溶解した溶液を、DL-乳酸重合体（重量平均分子量25,900、数平均分子量7,100、末端カルボキシル基量98.2 μ mol/g）3.85g および３－ヒドロキシー２－ナフトエ酸0.15g をジクロロメタン5.5ml およびエタノール0.35ml の混有機溶媒で溶解した溶液と混合してホモ

ジナイザーで乳化し、W／Oエマルションを形成した。その後の操作は実施例7に記載と同様にしてマイクロカプセル粉末を得た。マイクロカプセルの質量回収率は48.8%、マイクロカプセル中のペプチドB含量は21.31%、3－ヒドロキシー2－ナフトエ酸含量は2.96%であった。そしてこれらの実現含量を仕込み含量で除して求めた封入効率は、ペプチドBにおいて106.5%、3－ヒドロキシー2－ナフトエ酸において98.7%であった。

比較例2

ペプチドBの酢酸塩1.00gを1.00mlの蒸留水に溶解した溶液を、実施例12と同じDL-乳酸重合体4.00gをジクロロメタン5mlで溶解した溶液と混合してホモジナイザーで乳化し、W／Oエマルションを形成した。その後の操作は実施例7に記載と同様にしてマイクロカプセル粉末を得た。マイクロカプセルの質量回収率は48.7%、マイクロカプセル中のペプチドB含量は11.41%であった。そしてこの実現含量を仕込み含量で除して求めたペプチドBの封入率は57.1%であって、実施例12に比べてはるかに低い。従って3－ヒドロキシ2－ナフトエ酸の添加によりペプチドBの封入効率が上昇したことは明らかである。

実施例13

DL-乳酸重合体（重量平均分子量30,600、数平均分子量14,400、ラベル化定量法によるカルボキシル基量63.0 μmol/g）89.2gをジクロロメタン115.3gで溶解した溶液と、3－ヒドロキシー2－ナフトエ酸3.45gをジクロロメタン38.8gおよびエタノール6.27gの混有機溶媒で溶解した溶液を混合して28.5℃に調節した。この有機溶媒溶液から224gを量り取り、ペプチドBの酢酸塩22.3gを20mlの蒸留水に溶解して44.9℃に加温した水溶液と混合して5分間撹拌して粗乳化した後ホモジナイザーを用い、10,000rpm、5分間の条件にて乳化しW／Oエマルションを形成した。次いでこのW／Oエマルションを16.3℃に冷却

後に、予め 15℃に調節しておいた 0.1% (w/w)ポリビニルアルコール (EG-40、日本合成化学製) 水溶液 20 リットル中に 5 分間で注入し、 HOMOMIC LINE FLOW (特殊機化製) を用いて 7,000rpm で撹拌しW／O／Wエマルションとした。このW／O／Wエマルションを 15℃で 3 時間撹拌してジクロロメタンおよびエタノールを揮散ないしは外水相中に拡散させ、油相を固化させた後、75 μm の目開きの篩いを用いて篩過し、次いで遠心機 (H-600S, 国産遠心器製) を用いて 2,000rpm で連続的にマイクロカプセルを沈降させて捕集した。捕集されたマイクロカプセルは少量の蒸留水に再分散し、90 μm の目開きの篩いを用いて篩過した後マンニトール 9.98g を添加して溶解した後凍結乾燥して粉末として得られた。添加したマンニトールを計算で除外して求めたマイクロカプセルの質量回収率は 66.5%、マイクロカプセル中のペプチドＢ含量は 22.3%、３－ヒドロキシ－２－ナフトエ酸含量は 2.99%であった。そしてこれら実現含量を仕込み含量で除して求めた封入率は、ペプチドＢにおいて 104.5%、３－ヒドロキシ－２－ナフトエ酸において 102.1%であった。

実験例２

実施例８に記載のマイクロカプセル約 45mg を 0.3ml の分散媒 (0.15 mg のカルボキシメチルセルロース, 0.3mg のポリソルベート 80, 15mg のマンニトールを溶解した蒸留水) に分散して 7 週齢雄性 SD ラットの背部皮下に 22G 注射針で投与した。投与から所定時間後にラットを屠殺して投与部位に残存するマイクロカプセルを取り出し、この中のペプチドＢおよび３－ヒドロキシ－２－ナフトエ酸を定量してそれぞれの初期含量で除して求めた残存率および使用したＤＬ－乳酸重合体の特性を表２に示す。

表２

実施例８記載のマイクロカプセルのＤＬ－乳酸重合体の特性

Mw(Da)	25,200
[COOH](μ mol/g－ポリマー)	62.5

残存率：

	ペプチドＢ	３－ヒドロキシ－２－ナフトエ酸
１日	93.1%	91.0%
２週	84.2%	54.1%
４週	75.7%	34.5%
８週	63.0%	5.1%
１２週	46.9%	0.0%
１６週	31.7%	0.0%
２０週	24.0%	0.0%

表２から明らかなように、実施例８に記載のマイクロカプセルは生理活性物質を高含量に含んでいるのにも関わらず、投与後一日おける生理活性物質の残存率は９０％以上と飛躍的に高い。従って、３－ヒドロキシ－２－ナフトエ酸は徐放性製剤中に生理活性物質を高含量で取り込ませる効果だけでなく、生理活性物質の初期の過剰放出を非常によく抑止する効果を併せ持つのは明白である。そして、このマイクロカプセルは非常に長期にわたって生理活性物質を一定速度で放出させることを実現している。また１２週以降、３－ヒドロキシ－２－ナフトエ酸はマイクロカプセルから完全に消失しているが、生理活性物質の放出はそれまでと同じ一定速度を持続していて、徐放性製剤として有効であ

る。

実験例３

実施例７、９～１２および比較例１で得られた各マイクロカプセルを実験例２に記載と同様に投与ならびに回収したのち、この中のペプチドＢを定量してそれぞれの初期含量で除して求めた残存率および使用したＤＬ－乳酸重合体の特性を表３に示す。

表３

ＤＬ－乳酸重合体の特性：

	実施例７	実施例９	実施例１０	実施例１１	実施例１２	比較例１
Mw(Da)	36,000	28,800	49,500	19,900	25,900	28,800
[COOH](μmol/g－ポリマー)	70.4	78.1	45.9	104.6	98.2	78.1
残存率						
１日	92.9%	94.6%	93.0%	92.3%	89.4%	83.1%
２週	82.2%	82.2%	80.4%	37.5%	34.3%	73.0%
４週	69.6%	69.2%	58.3%	30.7%	29.7%	65.3%
８週	62.1%	56.0%	36.6%	24.6%	20.8%	
１２週	47.9%	39.4%	30.8%	18.6%		
１６週	32.2%		28.0%			
２０週	(測定せず)		22.9%			
２４週	11.6%					
２８週	4.1%					

表２および表３から明らかなように、実施例７～１２に記載のマイクロカプセ

ルの、投与後一日おける残存率はすべて約９０％ないしはそれ以上であり、比較例１のそれに比較して飛躍的に高い。従って、３－ヒドロキシ－２－ナフトエ酸は徐放性製剤中に生理活性物質を高含量で取り込ませる効果だけでなく、生理活性物質の初期の過剰放出を非常によく抑止する効果も併せ持つのは明白である。なかでも実施例７～９に記載のマイクロカプセルを用いた実験例より、生体内分解性ポリマーとして重量平均分子量が約２０，０００～約５０，０００でかつラベル化定量法によるカルボキシル基量が約５０～９０μｍｏｌ／ｇであるＤＬ－乳酸を用いた場合には、非常に長期にわたり生理活性物質を一定した速さで放出させることができる。

実験例４

実施例７で得られたマイクロカプセルを実験例２に記載の方法でラットに皮下投与した後、採血して得られた血清中のペプチドＢの濃度とテストステロン濃度を測定した結果を表４に示す。

表４

	12週	16週	24週	26週	28週
ペプチドＢ(ng/ml)	1.10	1.65	1.46	2.73	1.30
テストステロン(ng/ml)	0.18	0.45	0.68	0.41	0.71

表４から明らかなように生理活性物質の血中濃度は２８週後まで一定の値に維持されており、これはマイクロカプセルから生理活性物質が２８週にわたって持続的に放出されたことを意味している。そして、その期間中、薬効を示すテストステロン濃度は常に正常値レベル以下に抑制されており、製剤中に３－ヒドロキシ－２－ナフトエ酸を含有しても生理活性物質は、その活性を損なうことなく、長期にわたってマイクロカプセル中に安定に存在し、徐放されている

ことが明らかとなった。

実施例１４

強塩基性イオン交換カラム（SeP-Pak Plus QMAカートリッジ、ウォーターズ社製）に０．５Ｎ水酸化ナトリウム水溶液／メタノールの混液（v/v=1/5）を通して塩化物イオンを排出した。流出液が、硝酸酸性下で硝酸銀溶液を添加しても白濁しなくなった後、水／メタノールの混液（v/v=1/5）を通して過剰のアルカリを排出した。流出液が中性であることを確認した後、ペプチドＢの酢酸塩18.8mgを水／メタノールの混液（v/v=1/5）2mlに溶解して、上記前処理を施したカラムを通過させた。この流出液と、この後さらに混液のみを６ｍｌ通過させたものとを併せ、これに３－ヒドロキシ－２－ナフトエ酸５．９１mgを水／メタノールの混液（v/v=1/5）１mlに溶解したものを混合して、ロータリーエヴァポレーターで濃縮した。混合液に白濁を生じたら水２ｍｌを加えて撹拌し、遠心（３０００ｒｐｍ、２０℃、１５分）して上澄みを除去、さらに数回水洗を繰り返した後に沈殿を真空乾燥（４０℃、一夜）して、ペプチドＢの３－ヒドロキシ－２－ナフトエ酸塩４．０９ｍｇを得た。

この塩に水０．５ｍｌを加えて室温で４時間撹拌した後、液を０．２μmフィルターで濾過してＨＰＬＣで定量した。ペプチドＢおよび３－ヒドロキシ－２－ナフトエ酸の濃度はそれぞれ２．３７ｇ／Ｌ、０．７５１ｇ／Ｌであった。撹拌後も塩の一部は溶け残っており上記値はペプチドＢの３－ヒドロキシ－２－ナフトエ酸塩の水溶解度と考えられ、ペプチドＢの酢酸塩の水溶解度が１０００ｇ／Ｌ以上であるのに比較して１００分の１以下に低下している。このことは、ペプチドＢの３－ヒドロキシ－２－ナフトエ酸塩がペプチドＢの徐放性製剤として利用できることを示している。

産業上の利用可能性

本発明の徐放性組成物は生理活性物質を高含量で含有し、かつその初期過剰放出を抑制し長期にわたる安定した放出速度を実現することができる。

請 求 の 範 囲

1．生理活性物質またはその塩、ヒドロキシナフトエ酸またはその塩および生体内分解性ポリマーまたはその塩を含有してなる徐放性組成物。

2．生理活性物質が生理活性ペプチドである請求の範囲第１項記載の徐放性組成物。

3．生理活性物質がLH-RH誘導体である請求の範囲第２項記載の徐放性組成物。

4．ヒドロキシナフトエ酸が３－ヒドロキシ－２－ナフトエ酸である請求の範囲第１項記載の徐放性組成物。

5．生体内分解性ポリマーがα－ヒドロキシカルボン酸重合体である請求の範囲第１記載の徐放性組成物。

6．α－ヒドロキシカルボン酸重合体が乳酸－グリコール酸重合体である請求項の範囲第５項記載の徐放性組成物。

7．乳酸とグリコール酸の組成モル％が１００／０～４０／６０である請求の範囲第６項記載の徐放性組成物。

8．乳酸とグリコール酸の組成モル％が１００／０である請求の範囲第７項記載の徐放性組成物。

9．重合体の重量平均分子量が約３，０００～約１００，０００である請求の範囲第６項記載の徐放性組成物。

１０．重量平均分子量が約２０，０００～５０，０００である請求の範囲第９項記載の徐放性組成物。

１１．LH-RH誘導体が式

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z

[式中、YはDLeu、DAla、DTrp、DSer(tBu)、D2NalまたはDHis(ImBzl)を示し、ZはNH-C_2H_5またはGly-NH_2を示す。]で表されるペプチドである請求の範囲第３

項記載の徐放性組成物。

１２．重合体の末端のカルボキシル基量が重合体の単位質量（グラム）あたり５０－９０マイクロモルである請求の範囲第６項記載の徐放性組成物。

１３．ヒドロキシナフトエ酸またはその塩とLH-RH誘導体またはその塩のモル比が３対４ないし４対３である請求の範囲第３項記載の徐放性組成物。

１４．徐放性組成物中、LH-RH誘導体またはその塩が１４％(w/w)から２４％(w/w)含有される請求の範囲第１３項記載の徐放性組成物。

１５．生理活性物質またはその塩が微水溶性または水溶性である請求の範囲第１項記載の徐放性組成物。

１６．注射用である請求の範囲第１項記載の徐放性組成物。

１７．生理活性物質またはその塩、生体内分解性ポリマーまたはその塩およびヒドロキシナフトエ酸またはその塩の混合液から溶媒を除去することを特徴とする請求の範囲第１項記載の徐放性組成物の製造法。

１８．生体内分解性ポリマーまたはその塩およびヒドロキシナフトエ酸またはその塩を含有する有機溶媒溶液に生理活性物質またはその塩を混合、分散し、次いで有機溶媒を除去することを特徴とする請求の範囲第１７項記載の徐放性組成物の製造法。

１９．生理活性物質またはその塩が生理活性物質またはその塩を含有する水溶液である請求の範囲第１８項記載の徐放性組成物の製造法。

２０．生理活性物質の塩が遊離塩基または酸との塩である請求の範囲第１７項記載の製造法。

２１．請求の範囲第１項記載の徐放性組成物を含有してなる医薬。

２２．請求の範囲第３項記載の徐放性組成物を含有してなる前立腺癌、前立腺肥大症、子宮内膜症、子宮筋腫、子宮線維腫、思春期早発症、月経困難症もしくは乳癌の予防、治療剤または避妊剤。

２３．生理活性物質のヒドロキシナフトエ酸塩および生体内分解性ポリマーま

たはその塩を含有してなる徐放性組成物。

２４．ヒドロキシナフトエ酸またはその塩を用いることを特徴とする徐放性組成物からの生理活性物質の初期過剰放出を抑制する方法。

２５．ヒドロキシナフトエ酸またはその塩を用いることを特徴とする徐放性組成物への生理活性物質の封入効率を向上する方法。

２６．生理活性ペプチドのヒドロキシナフトエ酸塩。

２７．水溶性または微水溶性である請求の範囲第２６項記載の生理活性ペプチドのヒドロキシナフトエ酸塩。

２８．生理活性ペプチドのヒドロキシナフトエ酸塩を含有してなる徐放性組成物。

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/00086

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] A61K47/30, A61K47/12, A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] A61K47/30, A61K47/12, A61K37/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 9-315997, A2 (Takeda Chemical Industries,Ltd.), 9 December, 1997 (09. 12. 97) & WO, 9735563, A2 & AU, 9720432, A1 & EP, 889722, A2	1-28
A	JP, 8-259460, A2 (Takeda Chemical Industries,Ltd.), 8 October, 1996 (08. 10. 96) & WO, 9622786, A1 & AU, 9644591, A1	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 April, 1999 (21. 04. 99)	11 May, 1999 (11. 05. 99)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

国際調査報告

国際出願番号　PCT／JP99／00086

A.　発明の属する分野の分類（国際特許分類（IPC））

Int.Cl⁶　A61K47/30, A61K47/12, A61K37/02

B.　調査を行った分野

調査を行った最小限資料（国際特許分類（IPC））

Int.Cl⁶　A61K47/30, A61K47/12, A61K37/02

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

CAS ONLINE

C.　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
A	JP, 9-315997, A2 (武田薬品工業株式会社) 9．12月．1997 (09．12．97) &WO, 9735563, A2&AU, 9720432, A1&EP, 889722, A2	1－28
A	JP, 8-259460, A2 (武田薬品工業株式会社) 8．10月．1996 (08．10．96) &WO, 9622786, A1&AU, 9644591, A1	1－28

☐ C欄の続きにも文献が列挙されている。

☐ パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
「E」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」口頭による開示、使用、展示等に言及する文献
「P」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
21．04．99	11.05.99

国際調査機関の名称及びあて先	特許庁審査官（権限のある職員）	
日本国特許庁（ISA／JP） 郵便番号100－8915 東京都千代田区霞が関三丁目4番3号	鶴見　秀紀　印	4C　8415
	電話番号　03－3581－1101　内線　3452	

様式PCT／ISA／210（第2ページ）（1998年7月）

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/16, 9/50, 47/12, 38/00

A1

(11) International Publication Number: **WO 98/32423**

(43) International Publication Date: 30 July 1998 (30.07.98)

(21) International Application Number: PCT/JP98/00339

(22) International Filing Date: 28 January 1998 (28.01.98)

(30) Priority Data:
9/15203 29 January 1997 (29.01.97) JP

(71) Applicant *(for all designated States except US)*: TAKEDA CHEMICAL INDUSTRIES, LTD. [JP/JP]; 1–1, Doshomachi 4–chome, Chuo–ku, Osaka–shi, Osaka 541 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KAMEI, Shigeru [JP/JP]; 7–1–509, Sumiregaoka 1–chome, Takarazuka–shi, Hyogo 665 (JP). OHTA, Tsutomu [JP/JP]; 1–3, Satsukigaoka 5–chome, Ikeda–shi, Osaka 563 (JP). SAIKAWA, Akira [JP/JP]; 2–45, Nagaoka 2–chome, Nagaokakyo–shi, Kyoto 617 (JP). IGARI, Yasutaka [JP/JP]; 4–25–503, Motoyamaminamimachi 5–chome, Higashinada–ku, Kobe–shi, Hyogo 658 (JP).

(74) Agents: ASAHINA, Tadao et al.; Osaka Plant of Takeda Chemical Industries, Ltd., 17–85, Jusohonmachi 2–chome, Yodogawa–ku, Osaka–shi, Osaka 532 (JP).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SUSTAINED–RELEASE MICROSPHERES, THEIR PRODUCTION AND USE

(57) Abstract

The present invention provides a method of producing a sustained–release microsphere which comprises emulsification, a physiological active peptide and a pamoic acid by a biodegradable polymer; a sustained–release microsphere comprising an about 0.01 to about 10 μm particle size of a pamoic acid salt of physiologically active peptide and a biodegradable polymer; a sustained–release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer; and a sustained–release preparation comprising the microsphere. The microsphere contains a large amount of the physiologically active peptide and can regulate a release rate of the physiological peptide.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

DESCRIPTION

Sustained-Release Microspheres, Their Production and Use

TECHNICAL FIELD

The present invention relates to sustained-release microspheres comprising a physiologically active peptide, a sustained-release preparation comprising the microspheres, and a method of producing the microspheres.

BACKGROUND ART

For the preparation of physiologically active peptides as sustained-release microspheres, various methods have been reported so far. For example, Japanese Patent Unexamined Publication No. 97334/1995 discloses a sustained-release preparation comprising a physiologically active peptide possessing LH-RH antagonist activity or a salt thereof and a biodegradable polymer having a free carboxyl group at one end, and a method of its production.

Japanese Patent Unexamined Publication Nos. 121222/1989 and 66625/1991 describe a control release drug composition comprising a water-insoluble adduct salt of a water-soluble peptide such as an LH-RH derivative converted by using a non-toxic water-insoluble acid such as pamoic acid, tannic acid or stearic acid, or the like, and a polymer like a polylactide or a copolymer of lactic acid and glycolic acid, and a method of its production, suggesting that drug release duration can be prolonged by converting the drug to water-insoluble, as defined to have a solubility in distilled water of not more than 25 mg/l.

Japanese Patent Unexamined Publication No. 68511/1991 describes a method of producing a sustained-release microparticle formulation wherein a microparticles is formed by dispersing a drug solution into a polymer solution in which the drug compound is insoluble, followed by hardening of the resulting product, and a microparticle formulation of somatostatin or a derivative thereof

obtained by the method. It also suggests the use of a pamoic acid salt may enable to stabilize the somatostatin derivative (octreotide) in the microparticle formulation.

Japanese Patent Unexamined Publication No. 221855/1993 discloses a process for the production of a pharmaceutical composition for the sustained and controlled release of a peptide, obtained in the form of microsphere of a biodegradable polymeric material incorporating the peptide which comprises initially converting a water-soluble peptide into a water-insoluble peptide, followed by preparing an o/w emulsion, and extracting the organic solvent for the polymeric material in an excess of aqueous medium.

Furthermore, Japanese Patent Unexamined Publication No. 340543/1994 describes a sustained-release preparation wherein the embonic acid (pamoic acid) or ascorbic acid salt of a peptide as an active ingredient in a matrix of a polylactide having a lactide/glycolide molar ratio of 100:0 to 40:60, a molecular weight of 10,000 to 200,000, and a degree of polydispersion of 1.7 to 3.0, suggesting that embonic acid and ascorbic acid are useful as stabilizers in peptides in polylactides.

WO95/15767 describes the embonic acid salt (pamoic acid) of cetrorelix (LH-RH antagonist) and a method of its production, stating that the duration of action was about the same as that of peptide embonate in a biologically degradable polymer.

As stated above, it has been known that a pamoic acid salt of a physiologically active peptide in a formulation enables to stabilize the physiologically active peptide or to control its release; however, there have been absolutely no reports of a composition wherein a fine and minute pamoic acid salt of a physiologically active peptide is formed in the presence of a biodegradable polymer, or a three-component salt comprising a physiologically active

peptide, a pamoic acid and a biodegradable polymer, and a composition containing it.

In addition, the sustained-release preparations obtained by these published methods are unsatisfactory in view of clinical application.

After extensive investigation aiming at resolving the above problems, the present inventors found that a sustained-release microsphere comprising a physiologically active peptide at high contents, and capable of controlling its release rate, can be produced by emulsification of a solution of a physiologically active peptide and a solution of a pamoic acid or a salt thereof by a biodegradable polymer.

More specifically, the present inventors found that a physiologically active peptide can be incorporated at high contents by emulsification of a physiologically active peptide having basic groups capable of forming salts with a pamoic acid, a pamoic acid or a salt thereof and a biodegradable polymer in a molecular dispersion like in solution to form a fine pamoic acid salt of the physiologically active peptide of about 0.01 to about 10 μm in particle size not later than solvent removal, and producing a microsphere containing the pamoic acid salt, unlike conventional methods involves pre-conversion of physiologically active peptide to pamoic acid salt in the absence of polymer.

The present inventors also found in the case of a peptide having not less than 2 basic groups that a pamoic acid/physiologically active peptide ratio differing from that of microspheres produced by conventional methods which contain a previously prepared pamoic acid salt of a physiologically active peptide, and that the physiologically active peptide release rate can be controlled by the decomposition rate of the biodegradable polymer when the physiologically active peptide is allowed to form a complex or salt with both a pamoic acid and a

biodegradable polymer having a free carboxyl group, and a microsphere containing it is prepared.

After further investigations based on these findings, the inventors developed the present invention.

DISCLOSURE OF INVENTION

The present invention provides:

(1) A method of producing a sustained-release microsphere which comprises emulsification of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a pamoic acid or an alkaline metal salt thereof with a biodegradable polymer;

(2) The method according to (1), which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a solution of the pamoic acid or an alkaline metal salt thereof in solution of the biodegradable polymer with an organic solvent, and removing the solvent;

(3) The method according to (1), which comprises dissolving the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, the pamoic acid or an alkaline metal salt thereof and the biodegradable polymer in an organic solvent, and removing the solvent;

(4) The method according to (1), which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and the biodegradable polymer with an organic solvent and a solution of the pamoic acid or an alkaline metal salt thereof, and removing the solvent;

(5) The method according to (1), which comprises emulsification of a solution of the biodegradable polymer and the pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, and removing the solvent;

(6) The method according to any one of (2) to (5), wherein the removing of the solvent is conducted by in-water drying method;

(7) The method according to (6), which furthermore followed by freeze drying;

(8) The method according to any one of (2) to (5), wherein a concentration of the physiologically active peptide in the solution mixture is about 1 to about 25 wt% of the solution mixture;

(9) The method according to any one of (2) to (5), wherein a concentration of the biodegradable polymer in the solution mixture is about 1 to about 25 wt% of the solution mixture;

(10) The method according to any one of (2) to (5), wherein a concentration of the pamoic acid or a salt thereof in the solution mixture is about 0.05 to about 5 wt% of the solution mixture;

(11) The method according to (2) or (4), wherein the solution of the pamoic acid or a salt thereof is a methanol solution of the pamoic acid or a salt thereof;

(12) The method according to (4), wherein an amount of the solution of the pamoic acid or a salt thereof is about 2 to about 90 (v/v) % to the organic solvent of the physiologically active peptide and the biodegradable polymer in the of solution mixture;

(13) The method according to (1), wherein the physiologically active peptide or a salt thereof is a free base or a salt with a weak acid of not less than pKa4.0;

(14) The method according to (1), wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid;

(15) The method according to (1), wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid;

(16) The method according to (1), wherein the physiologically active peptide is an LH-RH agonist;

(17) The method according to (1), wherein the physiologically active peptide is an LH-RH antagonist;

(18) The method according to (1), the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof;

(19) The method according to (1), the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 acetate;

(20) The method according to (1), wherein the biodegradable polymer is a polymer of α-hydroxy carboxylic acids;

(21) The method according to (20), wherein the polymer of α-hydroxy carboxylic acids is a lactic acid/glycolic acid polymer;

(22) The method according to (21), wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%);

(23) The method according to (20), wherein a weight-average molecular weight of the biodegradable polymer is 3,000 to 100,000;

(24) The method according to (1), wherein the biodegradable polymer is a polylactic acid;

(25) The method according to (24), wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000;

(26) The method according to any one of (2) to (5), wherein the organic solvent is a dichloromethane;

(27) The method according to (1), wherein the physiologically active peptide is a peptide having one basic group capable of forming a salt with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising an about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide;

(28) The method according to (1), wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer;

(29) A sustained-release microsphere which is obtainable by the method according to (1);

(30) The sustained-release microsphere which comprises an about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide and a biodegradable polymer;

(31) A sustained-release microsphere which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer;

(32) A sustained-release microsphere which comprises not more than about 0.8 mol of pamoic acid to 1 mol of physiologically active peptide;

(33) The sustained-release microsphere according to (32), which comprises about 0.3 to about 0.7 mol of the pamoic acid to 1 mol of the physiologically active peptide is contained;

(34) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a physiologically active peptide having basic groups capable of forming salts with a weak acid of not less than pKa4.0;

(35) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid;

(36) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active

peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid;

(37) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is an LH-RH agonist;

(38) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is an LH-RH antagonist;

(39) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof;

(40) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 acetate;

(41) The sustained-release microsphere according to (28) or (30), wherein the biodegradable polymer is a polymer of α-hydroxy carboxylic acids;

(42) The sustained-release microsphere according to (41), wherein the polymer of α-hydroxy carboxylic acids is a lactic acid/glycolic acid polymer;

(43) The sustained-release microsphere according to (42), wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%);

(44) The sustained-release microsphere according to (41), wherein a weight-average molecular weight of the polymer is 3,000 to 100,000;

(45) The sustained-release microsphere according to any one of (29) to (32), wherein the biodegradable polymer is a polylactic acid;

(46) The sustained-release microsphere according to (45), wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000;

(47) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the

physiologically active peptide in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere;

(48) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the pamoic acid or a salt thereof in the sustained-release microsphere is about 0.1 to about 25 wt% of the sustained-release microsphere;

(49) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the biodegradable polymer in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere;

(50) The sustained-release microsphere according to (30), wherein a ratio of the about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide in the sustained-release microsphere is about 15 to about 90 wt% of the sustained-release microsphere;

(51) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof and a content of the peptide is about 15 to about 30 wt% to the total microcapsule;

(52) A sustained-release microsphere which is produced by the method according to (1);

(53) A sustained-release preparation which comprises the microsphere according to any one of (29) to (32);

(54) The sustained-release preparation according to (53), which is an injectable preparation;

(55) A sustained-release preparation which comprises the microsphere according to (37) or (38); and

(56) The sustained-release preparation according to (55), which is a treating or preventive agent for prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma,

dysmenorrhea, precocious puberty or breast cancer, or a contraceptive agent.

Detailed discription

The physiologically active peptides used in the method of the present invention may be any peptides which are capable of forming a salt with a pamoic acid and showing physiologically activities. Examples of the peptide are a peptide having about 300 to about 40,000, preferably about 400 to 30,000, furthermore preferably about 500 to 20,000 molecular weight, and so on.

Such peptides may preferably have basic groups which are capable of forming a salt with a weak acid of not less than pKa 4.0 (e.g. carbonic acid, bicarbonic acid, bornic acid, C_{1-3} lower alkane-monocarbonic acid, etc.).

When the physiologically active peptide has some basic groups in its molecule, so long as at least one basic group is capable of forming a salt with a pamoic acid, other groups may form salts. Physiologically active peptides having not only basic groups but also acidic groups which are free or forming salts may be involved in the physiologically active peptide of the present invention, so long as they are capable of forming salts with a pamoic acid.

The representative examples of activities of the physiologically peptides are hormone activity, etc.. The physiologically active peptides may be natural products, synthesized products, half-synthesized products or gene products, and furthermore may be analogs and/or derivatives thereof. The mechanism of these physiologically active peptides may be agonistic or antagonistic.

Examples of the physiologically active peptides include luteinizing hormone-releasing hormone (sometimes referred to as LH-RH, gonadotropin-releasing hormone or Gn-RH), insulin, somatostatin, somatostatin derivative (Sandostatin; see US Patent Nos. 4,087,390, 4,093,574,

4,100,117 and 4,253,998), growth hormones (GH), growth hormone-releasing hormones (GH-RH), prolactin, erythropoietin (EPO), adrenocorticotropic hormone (ACTH), ACTH derivatives (e.g., ebiratide), melanocyte-stimulating hormone (MSH), thyrotropin-releasing hormone (represented by the structural formula (Pyr)Glu-His-ProNH_2, hereinafter also referred to as TRH) and salts and derivatives thereof (see Japanese Patent Unexamined Publication Nos. 121273/1975 and 116465/1977), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), vasopressin, vasopressin derivative (desmopressin, see Folia Endocrinologica Japonica, Vol. 54, No. 5, pp. 676-691 (1978)), oxytocin, calcitonin, glucagon, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, enkephalin derivatives (see US Patent No. 4,277,394 and European Patent Publication No. 31567), endorphin, kyotorphin, interferons (e.g., α-, β- and γ-interferons), interleukins (e.g., interleukin 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12), tuftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor (THF), blood thymic factor (FTS) and derivatives thereof (see US Patent No. 4,229,438), tumor necrosis factor (TNF), colony-stimulating factors (e.g., CSF, GCSF, GMCSF, MCSF), motilin, dynorphin, bombesin, neurotensin, caerulein, bradykinin, atrium sodium-excretion increasing factor, nerve growth factor (NGF), cell growth factors (e.g., EGF, TGF-α, TGF-β, PDGF, acidic FGF, basic FGF), nerve nutrition factors (e.g., NT-3, NT-4, CNTF, GDNF, BDNF), and endothelin-antagonistic peptides and their analogs (derivatives) (see European Patent Publication Nos. 436189, 457195 and 496452, and Japanese Patent Unexamined Publication Nos. 94692/1991 and 130299/1991), a protein derived from α 1 domain of major histocompatibility class I antigen complex (Proceedings of the National Academy of Sciences of the United State of America, vol. 91,9086-9090

(1994) and vol. 94,11692-11697 (1997)) which has an activity of inhibiting an internalization of insulin receptor, insulin-like growth factor (IGF)-1 receptor, IGF-2 receptor, transferrin receptor, epidermal growth factor receptor, low density lipoprotein (LDL) receptor, macrophage scavenger receptor, GLUT-4 transporter, growth hormone receptor and leptin receptor, and their analogs (derivatives), furthermore their fragments or derivatives thereof.

When the physiologically active peptides are salts, the salts include pharmacologically acceptable salts. Examples of the salts are salts formed with inorganic acids (e.g., hydrochloric acid, sulfuric acid, nitric acid and bornic acid) or salts formed with organic acids (e.g., carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid and trifluoroacetic acid), when the physiologically active peptide has a basic group such as the amino group. Examples of the salts are salts formed with inorganic bases (e.g., alkaline metals such as sodium and potassium, alkaline earth metals such as calcium and magnesium) or salts formed with organic base compounds (e.g., organic amines such as triethylamine, and basic amino acids such as arginine), when the physiologically active peptide has an acidic group such as the carboxy group. And, the physiologically active peptide may form a metal complex compound (e.g., copper complex compound, zinc complex compound). Provided that, a pamoic acid salt of the physiologically active peptide is excluded from a salt of the physiologically active peptide used as a material for the method of production of the present invention.

Preferable physiologically active peptides for the present invention include, for example, LH-RH analogues effective against diseases dependent on LH-RH or hormones induced thereby, such as prostatic cancer, prostatic hypertrophy, endometritis, hysteromyoma, dysmenorrhea, precocious puberty and breast cancer, and as

contraceptives, and salts thereof, and somatostatin derivatives effective against diseases dependent on growth hormones and hormones induced thereby, and gastrointestinal diseases such as digestive ulcers, and salts thereof.

Specific examples of the LH-RH analogs or salts thereof are peptides described in Treatment with GnRH analogs: Controversies and perspectives, The Parthemon Publishing Group Ltd., 1996; and Japanese Patent Unexamined Publication Nos. 503165/1991, 101695/1991, 97334/1995 and 259460/1996 and so on.

The preferable examples of the physiologically active peptide having LH-RH antagonistic activity are a physiologically active peptide represented by the formula:

X-D2Nal-D4ClPhe-D3Pal-Ser-A-B-Leu-C-Pro-DAlaNH$_2$ (Ia)

wherein X is N(4H2-furoyl)Gly or NAc, A is a residue selected from NMeTyr, Tyr, Aph(Atz) and NMeAph(Atz), B is a residue selected from DLys(Nic), DCit, DLys(AzaglyNic), DLys(AzaglyFur), DhArg(Et$_2$), DAph(Atz) and DhCi, C is a residue selected from Lys(Nisp), Arg and hArg(Et$_2$), and so on.

In addition, the preferable examples of the physiologically active peptide having LH-RH antagonistic activity are physiologically active peptides described in US Patent No. 5,580,957 and so on. These peptides can be prepared by the methods described in the above-mentioned references or publications or similar methods.

The preferable examples of the physiologically active peptide having LH-RH agonistic activity are a physiologically active peptide represented by the formula:

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z (Ib)

wherein Y is a residue selected from DLeu, DAla, DTrp, Dser(tBu), D2Nal and DHis(1mBZl), Z is NH-C$_2$H$_5$ or Gly-NH$_2$, and so on. Of these peptides, the peptide wherein Y is DLeu and Z is NH-C$_2$H$_5$ is preferred. These peptides can be prepared by the methods described in the above-mentioned references or publications or similar methods.

Specific examples of the somatostatin derivatives or a salt thereof are described in Proceedings of National Academy of Science, USA, 93, 12513-12518, 1996 or references cited.

And, examples of the somatostatin derivatives which are selectively useful for cancer are

DPhe-Cys-Tyr-DTrp-Lys-Cys-ThrNH_2 (US Patent No. 5,480,870, European Patent Publication No. 50568) and so on.

Other preferable examples of the somatostatin derivatives are sandostatin (US Patent Nos. 4,087,390, 4,093,574, 4,100,117 and 4,253,998.) and so on.

Preferable examples of the physiologically active peptides having one basic group capable of forming a salt with a pamoic acid are a physiologically active peptide or a salt thereof, represented by the formula [Ib] having a LH-RH agonistic activity and so on.

Preferable examples of the physiologically active peptides having not less than 2 basic groups capable of forming salts with a pamoic acid are a physiologically active peptide, or a salt thereof, represented by the formula (Ia) having a LH-RH antagonistic activity. And, the physiologically active peptide represented by the formula (Ib) can be also used.

Particularly, more preferably examples of the physiologically active peptide are 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof (particularly, acetate, etc.).

Abbreviations used in the present specification are defined as follows:

N(4H2-furoyl)Gly	: N-tetrafuroyl glycine residue
NAc	: N-acetyl group
D2Nal	: D-3-(2-naphtyl)alanine residue
D4ClPhe	: D-3-(4-chlorophenyl)alanine residue
D3Pal	: D-3-(3-pyridyl)alanine residue
NMeTyr	: N-methyl tyrosine residue

Aph(Atz)	: N-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
NMeAph(Atz)	: N-methyl-[5'-(3'-amino-1'H-1',2'4'-triazolyl)]phenylalanine residue
DLys(Nic)	: D-(epsilon-N-nicotinoyl)lysine residue
DCit	: D-citrulline residue
DLys(AzaglyNic)	: D-(azaglycyl nicotinoyl)lysine residue
DLys(AzaglyFur)	: D-(azaglycyl furanyl)lysine residue
DhArg(Et_2)	: D-(N,N'-diethyl)homoarginine residue
DAph(Atz)	: D-N-[5'(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
DhCi	: D-homocitrulline residue
Lys(Nisp)	: (epsilon-N-isopropyl)lysine residue
hArg(Et_2)	: (N,N'-diethyl)homoarginine residue
DSer(tBu)	: D-(O-t-butyl)serine residue
DHis(1mBzl)	: D-(π-benzyl)histidine residue

Abbreviations for other amino acids are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature (European Journal of Biochemistry, 138, 9-37, 1984) or abbreviations in common use in relevant fields. When an optical isomer may be present in amino acids, it is of the L-configuration, unless otherwise stated.

Examples of the biodegradable polymers used for the method of the present invention include homopolymers and copolymers, which are synthesized from one or more α-hydroxy acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid), hydroxydicarboxylic acids (e.g., malic acid), hydroxytricarboxylic acids (e.g., citric acid) etc., mixtures thereof; poly-α-cyanoacrylates; polyamino acids (e.g., poly-γ-benzyl-L-glutamic acid) and maleic anhydride copolymers (e.g., styrene-maleic acid copolymers).

With respect to the above-described biodegradable polymer, copolymerization may be of the random, block or

graft type. When the above-mentioned α-hydroxy acids, hydroxydicarboxylic acids and hydroxytricarboxylic acids have an optical active center in their molecular structures, they may be of the D-, L- or DL-configuration. Of them, a lactic acid/glycolic acid polymer and a poly-α-cyanoacrylates are preferred, and furthermore a lactic acid/glycolic acid polymer is more preferred.

The biodegradable polymer is preferably (1) a biodegradable polymer consisting of a mixture of (A): a copolymer of a glycolic acid and a hydroxycarboxylic acid represented by the formula:

(II)

wherein R represents an alkyl group having 2 to 8 carbon atoms and (B): a polylactic acid or (2) a copolymer of lactic acid and glycolic acid.

With respect to the formula (II) above, the straight-chain or branched alkyl group represented by R, which has 2 to 8 carbon atoms, is exemplified by ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, tert-pentyl, 1-ethylpropyl, hexyl, isohexyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl and 2-ethylbutyl. Preferably, a straight-chain or branched alkyl group having 2 to 5 carbon atoms is used. Such alkyl groups include ethyl, propyl, isopropyl, butyl and isobutyl. More preferably, R is ethyl.

The hydroxycarboxylic acid represented by the formula (II) is exemplified by 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid, 2-hydroxyisocaproic acid and 2-hydroxycapric acid, with preference given to 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid and 2-hydroxycaproic acid, with greater preference given to 2-hydroxybutyric acid. Although the hydroxycarboxylic acid may be of the D-, L- or D,L-

configuration, it is preferable to use a mixture of the D- and L-configurations wherein the ratio of the D-/L-configuration (mol%) preferably falls within the range from about 75/25 to about 25/75, more preferably from about 60/40 to about 40/60, and still more preferably from about 55/45 to about 45/55.

With respect to the copolymer of glycolic acid and a hydroxycarboxylic acid represented by the formula (II) (hereinafter referred to as glycolic acid copolymer), copolymerization may be of random, block or graft type. A random copolymer is preferred.

The hydroxycarboxylic acid represented by the formula (II) may be a mixture of one or more kinds in a given ratio.

With respect to the composition ratio of glycolic acid and the hydroxycarboxylic acid represented by the formula (II) in glycolic acid copolymer (A), it is preferable that glycolic acid account for about 10 to about 75 mol% and hydroxycarboxylic acid for the remaining portion. More preferably, glycolic acid accounts for about 20 to about 75 mol%, and still more preferably about 40 to about 70 mol%. The weight-average molecular weight of the glycolic acid copolymer is normally 2,000 to 100,000, preferably 3,000 to 80,000, and more preferably 5,000 to 50,000. The polydispersity (weight-average molecular weight/number-average molecular weight) of the glycolic acid copolymer is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The above-described glycolic acid copolymer (A) can be produced by a known process, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

Although the above-described polylactic acid may be of the D- or L-configuration or a mixture thereof, it is preferable that the ratio of the D-/L-configuration (mol%) falls within the range from about 75/25 to about 20/80. The ratio of the D-/L-configuration (mol%) is more

preferably about 60/40 to about 25/75, and still more preferably about 55/45 to about 25/75. The weight-average molecular weight of said polylactic acid is preferably 1,500 to 100,000, more preferably 2,000 to 80,000, and still more preferably 10,000 to 60,000 (more preferably 15,000 to 50,000). Also, the dispersity of the polylactic acid is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

For producing a polylactic acid, two methods are known: ring-opening polymerization of lactide (a cyclic dimer of lactic acid) and polycondensation of lactic acid.

Glycolic acid copolymer (A) and polylactic acid (B) are used in a mixture wherein the (A)/(B) ratio (% by weight) falls within the range from about 10/90 to about 90/10. The emulsification ratio (% by weight) is preferably about 20/80 to about 80/20, and more preferably about 30/70 to about 70/30.

If either component (A) or (B) is in excess, the preparation obtained shows a drug release pattern not much different from that obtained with the use of component (A) or (B) alone; the linear release pattern which is obtainable with the mixed matrices cannot be expected in the last stage of drug release. Although the decomposition/elimination rate of glycolic acid copolymer (A) and polylactic acid varies widely, depending on molecular weight or composition, drug release duration can be extended by increasing the molecular weight of polylactic acid mixed or lowering the emulsification ratio (A)/(B), since the decomposition/elimination rate of glycolic acid copolymer (A) is usually higher. Conversely, drug release duration can be shortened by decreasing the molecular weight of polylactic acid mixed or increasing the emulsification ratio (A)/(B). Drug release duration can also be adjusted by altering the kind and content ratio of hydroxycarboxylic acid represented by the formula (II).

When the biodegradable polymer used is a polylactic acid or lactic acid/glycolic acid polymer, its composition ratio (lactic acid/glycolic acid) (mol%) is about 100/0 to about 40/60, preferably about 100/0 to about 45/55, more preferably about 100/0 to about 50/50.

The weight-average molecular weight of the above-described lactic acid/glycolic acid polymer is preferably about 3,000 to about 100,000, more preferably about 5,000 to about 80,000.

The dispersity of the lactic acid/glycolic acid polymer is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The decomposition/elimination rate of a lactic acid/glycolic acid polymer varies widely, depending on composition or molecular weight. Drug release duration can be extended by lowering the glycolic acid ratio or increasing the molecular weight, since decomposition/elimination is usually delayed as the glycolic acid ratio decreases. Conversely, drug release duration can be shortened by increasing the glycolic acid ratio or decreasing the molecular weight. To obtain a long-term (e.g., 1 to 6 months, preferably 1 to 4 months) sustained-release preparation, it is preferable to use a lactic acid/glycolic acid polymer whose composition ratio and weight-average molecular weight fall in the above-described ranges. With a lactic acid/glycolic acid polymer that decomposes more rapidly than that whose composition ratio and weight-average molecular weight fall in the above ranges, initial burst is difficult to suppress. On the contrary, with a lactic acid/glycolic acid polymer that decomposes more slowly than that whose composition ratio and weight-average molecular weight fall in the above ranges, it is likely that no effective amount of drug is released during some period.

Weight-average molecular weight, number-average molecular weight and dispersity, as defined herein, are

polystyrene-based molecular weights and dispersity determined by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, respectively. Measurements were taken using a GPC column KF804L × 2 (produced by Showa Denko) and an RI monitor L-3300 (produced by Hitachi, Ltd.), with chloroform as a mobile phase. Also, number-average molecular weight was calculated by dissolving the biodegradable polymer in an acetone-methanol mixed solvent, and titrating this solution with an alcoholic solution of potassium hydroxide with phenolphthalein as an indicator, to determine the terminal carboxyl group content. This molecular weight is hereinafter referred to as number-average molecular weight based on terminal group titration.

While the number-average molecular weight based on terminal group titration is an absolute value, the number-average molecular weight based on GPC measurement is a relative value that may vary depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width chosen, baseline chosen); it is therefore difficult to have an absolute numerical representation of the latter. In the case of a polymer having a free carboxyl group at one end, synthesized from lactic acid and glycolic acid by the catalyst-free polycondensation method, for example, however, the number-average molecular weight based on GPC measurement and that based on terminal group titration almost agree with each other. This fact for the lactic acid-glycolic acid polymer means that the number-average molecular weight based on terminal group titration falls within the range from about 0.5 to about 2 times, preferably from about 0.7 to about 1.5 times, the number-average molecular weight based on GPC measurement.

The lactic acid-glycolic acid polymer for the present invention can be produced by catalyst-free poly-

condensation from lactic acid and glycolic acid (Japanese Patent Unexamined Publication No. 28521/1986), or ring-opening polymerization from lactide, glycolide etc. using a catalyst (Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials, Volume 2, Marcel Dekker, Inc., 1995). Although the polymer synthesized by ring-opening polymerization is usually a polymer having no carboxyl groups, a polymer obtained by chemically treating the above-described polymer to provide a terminal free carboxyl group (Journal of Controlled Release, Vol. 41, pp. 249-257, 1996) can also be used.

The above-described lactic acid-glycolic acid polymer having a free carboxyl group at one end can be readily produced by known methods (e.g., catalyst-free poly-condensation method, see Japanese Patent Unexamined Publication No. 28521/1986), and a polymer having free carboxyl groups at unspecified position can be produced by known production methods (e.g., see WO94/15587 Publication).

Also, the lactic acid-glycolic acid polymer with a free carboxyl group at one end by chemical treatment after ring-opening polymerization is commercially available from Boehringer Ingelheim KG, for example.

Examples of the pamoic acid or a salt thereof may be a commercially available pamoic acid or a salt thereof. Examples of salts are alkaline metal salts (e.g. sodium salt, potassium salt, etc.), alkaline earth metal salts (e.g. calcium salt, magnesium salt, etc.), transition metal (e.g. zinc, iron, copper etc.) and so on. Particularly, alkaline metal salts such as sodium salt are preferred.

Examples of the solvents used for dissolution of the pamoic acid or a salt thereof and dissolution of the physiologically active peptide are water, alcohols (e.g. methanol, ethanol, etc.), pyridine, dimethylacetamide, acetic acid and so on. Preferable example is alcohols such as methanol and so on.

Examples of the organic solvents used for dissolution of the physiologically active peptide, pamoic acids or a salt and biodegradable polymer include halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride, etc.), ethers (e.g., ethyl ether, isopropyl ether, etc.), fatty acid esters (e.g., ethylacetate, butylacetate), aromatic hydrocarbons (e.g., benzene, toluene, xylene, etc.) and alcohols (e.g. methanol, ethanol, etc.) with preference given to halogenated hydrocarbons, particularly dichloromethane.

The production method of the present invention is characterized in producing a sustained-release microsphere comprising (i) an about 0.01 to about 10 μm particle size of a fine pamoic acid salt of the physiologically active peptide or (ii) a complex or salt formed by the physiologically active peptide, pamoic acid or a salt thereof and biodegradable polymer, wherein the fine pamoic acid salt and the complex or salt are formed by emulsification of a physiologically active peptide and a pamoic acid or a salt thereof with a biodegradable polymer, without preforming a pamoic acid salt of the physiologically active peptide in the absence of a biodegradable polymer as conducted in the past methods.

Therefore, the mixing method of the physiologically active peptide, biodegradable polymer and pamoic acid or a salt thereof is not limited, so long as the pamoic acid salt is not formed in the absence of the biodegradable polymer.

Thus, the present invention specifically provides:

(1) A method comprising emulsification of a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a solution of a pamoic acid or an alkaline metal salt thereof in a solution of a biodegradable polymer with an organic solvent, and removing the solvent;

(2) A method comprising dissolving a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, a pamoic acid or an alkaline metal salt thereof and a biodegradable polymer in an organic solvent, and removing the solvent;

(3) A method comprising emulsification of a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a biodegradable polymer with an organic solvent and a solution of a pamoic acid or an alkaline metal salt thereof, and removing the solvent; and

(4) A method comprising emulsification of solution of a biodegradable polymer and pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, and removing the solvent.

In the production method of the present invention, a concentration of the physiologically active peptide in the solution mixture is usually about 1 to about 25 wt%, preferably about 2 to about 20 wt% of the solution mixture.

A concentration of the biodegradable polymer in the solution mixture is usually about 1 to about 25 wt%, preferably about 2 to about 20 wt% of the solution mixture.

A concentration of the pamoic acid or a salt thereof in the solution mixture is usually about 0.05 to about 5 wt%, preferably about 0.2 to about 4 wt% of the solution mixture.

An amount of the solution of the pamoic acid or a salt thereof is usually about 2 to about 90 (v/v) % to the solution of the physiologically active peptide and the biodegradable polymer with an organic solvent.

For removing the solvent, in-water drying method, phase separation method and spray drying method are used.

The production method of the present invention is described specifically for each organic solvent removal method.

(I) In-water drying method:

A physiologically active peptide (including its salt) is added to solution of a biodegradable polymer with an organic solvent to yield a solution of the physiologically active peptide and the biodegradable polymer.

Examples of the organic solvent are halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate) and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in combination. The organic solvent used is preferably a halogenated hydrocarbon, more preferably dichloromethane.

Also, when a sufficient amount of physiologically active peptide is soluble in a solvent (e.g., water, alcohols (e.g., ethanol, methanol), acetonitrile, acetic acid) in a volume within 60% of the entire volume of the solution of the biodegradable polymer, a solution of the physiologically active peptide may be added to the solution of the biodegradable polymer to yield a solution of both, or the physiologically active peptide solution is emulsified in the solution of the biodegradable polymer to yield an o/o or w/o emulsion. In these procedures, it is undesirable to precipitate the physiologically active peptide.

Although the concentration of the biodegradable polymer used here in the solution varies, depending on the molecular weight of biodegradable polymer used, the kind of organic solvent, etc., it is normally chosen over the range from about 0.5 to about 70% (w/w), preferably about 1 to about 60% (w/w), and most preferably about 2 to about 50%

(w/w), when dichloromethane, for example, is used as the organic solvent.

The physiologically active peptide is normally added at about 30 mg to about 500 mg, preferably about 40 mg to about 400 mg, per ml of the above-described organic solvent in the biodegradable polymer solution.

Next, to the solution or o/o or w/o emulsion of the physiologically active peptide and biodegradable polymer, a solution of a pamoic acid or a pamoate (e.g., alkaline metal salts (sodium salt, potassium salt etc.), alkaline earth metal salts (e.g., calcium salt, magnesium salt) or salts with transition metals (e.g., zinc, iron, copper) (the solvent exemplified by water, alcohols (e.g., methanol, ethanol), pyridine, and dimethylacetamide) is added under emulsification by a known method such as the use of a homogenizer or ultrasonication.

Alternatively, when the physiologically active peptide or a salt thereof and the pamoic acid or a salt thereof, and furthermore the pamoic acid salt of the physiologically active peptide are completely soluble in an organic solvent (e.g., alcohols (methanol, ethanol etc.)), this organic solvent solution is added to the organic solvent solution of the biodegradable polymer under emulsification by a known method such as the use of a homogenizer or ultrasonication.

Although the pamoic acid or pamoate concentration in the solution in the above-described two addition methods is not subject to limitation, as long as it does not exceed the saturation concentration, it is preferably the saturation concentration, the ratio by volume of the pamoic acid or pamoate solution to the biodegradable polymer solution is preferably about 2 to about 90%, more preferably about 5 to about 70%, and most preferably about 10 to about 50%.

Next, the thus-obtained solution of the biodegradable polymer containing the physiologically active peptide and

pamoic acid (oil phase) is added to the second water phase to form an o (oil phase)/w (water phase) emulsion, after which the solvent in the oil phase is evaporated to yield microspheres. The volume of the water phase is normally chosen over the range from about 1 to about 10,000 times, preferably from about 2 to about 5,000 times, and most preferably from about 5 to about 2,000 times, that of the oil phase.

An emulsifier may be added to the above-described external water phase. The emulsifier may be any one, as long as it is capable of forming a stable o/w emulsion. Such emulsifiers include, for example, anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate), nonionic surfactants (e.g., polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals)), polyvinyl pyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These emulsifiers may be used singly or in combination. The emulsifier is preferably used at concentrations within the range from about 0.01% to about 10% (w/w), more preferably from about 0.05% to about 5% (w/w).

An osmolarity regulator may be added to the above-described external water phase. The osmolarity regulator may be any one, as long as it provides an osmolarity when prepared as an aqueous solution.

Examples of the osmolarity regulators are polyhydric alcohols, monohydric alcohols, monosaccharides, disaccharides, oligosaccharides or derivatives thereof.

Such polyhydric alcohols include, for example, dihydric alcohols such as glycerol, pentahydric alcohols such as arabitol, xylitol and adonitol, and hexahydric alcohols such as mannitol, sorbitol and dulcitol. Of these alcohols, hexahydric alcohols are preferred, with greater preference given to mannitol.

Such monohydric alcohols include, for example, methanol, ethanol and isopropyl alcohol, with preference given to ethanol.

Such monosaccharides include, for example, pentoses such as arabinose, xylose, ribose and 2-deoxyribose, and hexoses such as glucose, fructose, galactose, mannose, sorbose, rhamnose and fucose, with preference given to hexoses.

Such oligosaccharides include, for example, trisaccharides such as maltotriose and raffinose, and tetrasaccharides such as stachyose, with preference given to trisaccharides.

Derivatives of such monosaccharides, disaccharides and oligosaccharides include, for example, glucosamine, galactosamine, glucuronic acid and galacturonic acid.

These osmolarity regulators may be used singly or in combination.

These osmolarity regulators are used at concentrations such that the osmolarity of the external water phase is about 1/50 to about 5 times, preferably about 1/25 to about 3 times, that of physiological saline.

Organic solvent removal can be achieved by known methods, including the method in which the organic solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the organic solvent is evaporated, while the degree of vacuum is adjusted.

The thus-obtained microspheres (also referred to as microcapsules) are collected by centrifugation or filtration, after they are repeatedly washed with several additions of distilled water to remove the physiologically active peptide, pamoic acid, drug support, emulsifier etc. adhering to the microsphere surface, again dispersed in distilled water etc. and freeze-dried.

To prevent mutual aggregation of particles during the production process, an anticoagulant may be added. The

anticoagulant is exemplified by water-soluble saccharides such as mannitol, lactose, glucose and starches (e.g., corn starch), and proteins such as glycine, fibrin and collagen. The anticoagulant is preferably mannitol.

Also, if necessary after freeze-drying, the microspheres may be heated under reduced pressure under conditions that do not cause their mutual fusion to remove the water and organic solvent therefrom. In this case, it is preferable that the microspheres be heated at a temperature slightly higher than the midpoint of glass transition temperature of the biodegradable polymer, as obtained using a differential scanning calorimeter when the temperature is elevated at a rate of 10 to 20°C per minute. More preferably, the microspheres are heated within the temperature range from the midpoint of glass transition temperature of the biodegradable polymer to a temperature higher by about 30°C than the glass transition temperature. When a lactic acid-glycolic acid polymer is used as the biodegradable polymer, in particular, the microspheres are heated within the temperature range from the midpoint of glass transition temperature to a temperature higher by 20°C than the glass transition temperature, preferably within the temperature range from the midpoint of glass transition temperature to a temperature higher by 10°C than the glass transition temperature.

Although heating time varies, depending on the amount of microspheres and other factors, it is generally preferable that heating time be about 12 to about 168 hours, more preferably about 48 to 120 hours after the microspheres reach a given temperature. Heating time is most preferably about 48 hours to about 96 hours.

Any heating method can be used, as long as microspheres are uniformly heated.

Preferable thermal drying methods include, for example, the method in which thermal drying is conducted in a thermostated chamber, fluidized bed chamber, mobile phase

or kiln, and the method using microwaves for thermal drying. Of these methods, the method in which thermal drying is conducted in a thermostated chamber is preferred.

(II) The phase separation method:

For producing microspheres by the phase separation method, a coacervating agent is gradually added to the oil phase described in the above (I) under stirring, to precipitate and solidify the biodegradable polymer. The volume of the coacervating agent is about 0.01 to about 1,000 times, preferably about 0.05 to about 500 times, more preferably about 0.1 to about 200 times to the volume of the oil phase.

Any coacervating agent can be used, as long as it is a polymeric, mineral oil or vegetable oil compound miscible with the solvent for the biodegradable polymer and that does not dissolve the biodegradable polymer. Such coacervating agents include silicon oil, sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil, mineral oil, n-hexane and n-heptane. These may be used in combination of two or more kinds.

The thus-obtained microspheres are filtered to separate them, after which they are repeatedly washed with hexane, heptane etc. and heated to remove the coacervating agent. If necessary, in the same manner as with the above-described in-water drying method, miocrospheres are washed with distilled water several times repeatedly to remove the free drug, drug-retaining substance etc. adhering to the microsphere surface.

(III) Spray drying method:

For producing microspheres by this method, the oil phase described in in-water drying method (I) above is sprayed via a nozzle into the drying chamber of a spray drier to volatilize the organic solvent in the fine droplets in a very short time, to yield microsphere. The nozzle is exemplified by the double-fluid nozzle, pressure nozzle and rotary disc nozzle. The microspheres may be

then freeze-dried and thermally dried as necessary after being washed in the same manner as that described in in-water drying method (I).

For a dosage form other than the above-described microspheres, the oil phase described in in-water drying method (I) for microsphere production may be dried by evaporating the organic solvent and water, while the degree of vacuum is adjusted, followed by milling with a jet mill or the like to yield a fine powder.

The milled fine powder may be then freeze-dried and thermally dried after being washed in the same manner as that described in in-water drying method (I) for microsphere production.

The microspheres or fine powder can be orally or non-orally administered as such or in the form of various dosage forms prepared using them as a starting material. Specifically, they can be administered as muscular, subcutaneous, visceral and other injectable preparations or implant preparations, nasal, rectal, uterine and other transdermal preparations, oral preparations (e.g., solid preparations such as capsules (e.g., hard capsules, soft capsules), granules and powders; liquids such as syrups, emulsions and suspensions) etc.

For example, microspheres or a fine powder can be prepared as injectable formulations by suspending in water with a dispersing agent (e.g, surfactants such as Tween 80 and HCO-60, polysaccharides such as carboxymethyl cellulose and sodium alginate), a preservative (e.g., methyl paraben, propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose, proline) etc. to yield an aqueous suspension, or by dispersing in a vegetable oil such as sesame oil or corn oil to yield an oily suspension, whereby a practically useful sustained-release injectable preparation is obtained.

When the microspheres or fine powder is used in the form of an injectable suspension, their mean particle diameter is chosen over the range from about 0.1 to about 300 μm, as long as the requirements concerning the degree of dispersion and needle passage are met. Preferably, the mean particle diameter is about 1 to about 150 μm, more preferably about 2 to about 100 μm.

The microspheres or fine powder can be prepared as a sterile preparation by such methods as the method in which the entire production process is aseptic, the method using gamma rays for sterilization, and the method in which a preservative is added, which methods are not to be construed as limitative.

The term microsphere, as defined herein, is any microparticle containing a physiologically active peptide and a biodegradable polymer. The microsphere is preferably nearly spherical. Such microparticles include, for example, microcapsules containing one drug core in each particle, a multiple-core microcapsule containing a large number of drug cores in each particle, and microparticles wherein a drug in the molecular form is dissolved or dispersed as a solid solution in a matrix.

The sustained-release microsphere of the present invention can be produced by the above-described production method of the present invention. For example, when a physiologically active peptide having basic groups capable of forming salts with a pamoic acid (particularly, a physiologically active peptide having the basic group) is used in the above production method, sustained-release microspheres comprising an about 0.01 to about 10μm particle size of fine pamoic acid salt of the physiologically active peptide and a biodegradable polymer can be produced, whereby a physiologically active peptide is incorporated in microspheres at higher efficiencies than in the conventional microsphere production method, wherein

a pamoic acid salt of a physiologically active peptide is formed in advance, then mixed with a biodegradable polymer to yield microspheres comprising the pamoic acid salt of the physiologically active peptide, to enable the production of microspheres containing a physiologically active peptide at high contents.

On the other hand, when using a physiologically active peptide having two or more basic groups capable of forming salts with a pamoic acid and a biodegradable polymer having a free carboxyl group, sustained-release microspheres comprising a similarly fine complex or salt formed with the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer, can be produced. Here, the salt comprising three components may be any one, as long as the physiologically active peptide is incorporated between the pamoic acid and biodegradable polymer via a reversible bond, and may be a salt belonging to ortho salts, acidic salts, basic salts, complex salts, chelate salts etc. Complexes possibly formed by these three components are also included in the terminology of the above mentioned salts. The salt can be formed by emulsification of the three components in a molecular dispersion or similar state (e.g., solution state). In this case, the microsphere of the present invention, which contains the salts of three components, is characterized in that the pamoic acid/physiologically active peptide molar ratio therein is evidently smaller than that in the microspheres obtained by the conventional method; this demonstrates that the salt is a new salt differing from the salt in the conventional microsphere, which produces using a pamoic acid salt of a physiologically active peptide formed in the absence of a biodegradable polymer.

The sustained-release microspheres of the present invention contain a physiologically active peptide at high contents; the physiologically active peptide release from the preparation depends on the dissociation and dissolution

properties of the physiologically active peptide and/or a pamoic acid thereof, and the decomposition rate of the biodegradable polymer.

Thus, the present invention provides:

(1) A sustained-release microsphere (A) which comprises an about 0.01 to about 10 μm particle size of a pamoic acid salt of a physiologically active peptide and a biodegradable polymer; and

(2) A sustained-release microsphere (B) which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

Each of the sustained-release microspheres (A) and (B) is a sustained-release microsphere which comprises not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiologically active peptide.

In the microsphere (A) of the present invention, examples of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer are same those as mentioned above.

Preferable examples of the physiologically active peptide are a physiologically active peptide having groups capable of forming salts with a pamoic acid, particularly a physiologically active peptide having one basic group. And, preferable examples of the physiologically active peptide are LH-RH agonist represented by the formula (Ib), and particularly a compound C: 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 as shown in Example 11 as mentioned below, and so on.

Examples of the biodegradable polymer are a polylactic acid and a polymer of α-hydroxy carboxylic acids, and particularly a polylactic acid is prefered.

When the polylactic acid is used, the weight-average molecular weight is, for example, 10,000 to 60,000, more preferably 15,000 to 50,000.

As a composition ratio of lactic acid/glycolic acid is preferably 100/0 to 40/60 (mol%). Preferable weight-average molecular weight of the polymer is 5,000 to 80,000.

The particle size of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (A) of the present invention is usually about 0.01 to about 10 μm, preferably about 0.02 to about 5 μm, more preferably about 0.02 to about 4 μm.

In the sustained-release microsphere (A), the pamoic acid is usually included at the ratio of not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably about 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiological active peptide.

Although the emulsification ratio of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer in the sustained-release microsphere (A) may vary depending on kind of the physiologically active peptide, desired pharmacological action, duration of action and other factors, a ratio of the physiologically active peptide is usually not less than 15 wt%, preferably about 15 to about 85 wt%, more preferably about 20 to about 80 wt%, furthermore preferably about 20 to about 50 wt% to the total microspheres. Particularly, when the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof (particularly, acetate), a content of the peptide is preferably about 15 to about 30 wt%.

A ratio of the pamoic acid or a salt thereof in the sustained-release microsphere (A) is usually about 0.1 to about 25 wt%, preferably about 0.5 to about 15 wt%, more preferably about 1 to about 10 wt% to the microsphere.

A ratio of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (A) is usually about 15 (or about 15.1) to about 95 wt%, preferably about 20 to about 90 wt% to the sustained-release microspheres.

A ratio of the biodegradable polymer is usually about 15 to 85 wt%, preferably about 30 to about 60 wt% to the sustained-release microspheres.

In the microsphere (A) of the present invention, although the physiologically active peptides usually form salts with a pamoic acid, a part of the physiologically active peptides may exist without forming the salts.

In the microsphere (B) of the present invention, examples of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer are same those as mentioned above.

Preferable examples of the physiologically active peptide are a physiologically active peptide having not less than 2 basic groups capable of forming salts with a pamoic acid. Of these peptides, preferable examples of the physiologically active peptide are a LH-RH antagonist represented by the formula (Ia), and particularly a compound A as shown in Example 1 as mentioned below, and so on.

Examples of the biodegradable polymer are a polymer of α-hydroxy carboxylic acids, and particularly a lactic acid/glycolic acid polymer is prefered. As a composition ratio of lactic acid/glycolic acid is preferably 100/0 to 40/60 (mol%). Preferable weight-average molecular weight of the polymer is 5,000 to 80,000.

The particle size of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (B) of the present invention is usually about 0.01 to about 10 μm, preferably about 0.02 to about 5 μm, more preferably about 0.02 to about 4 μm.

In the sustained-release microsphere (B), the pamoic acid is usually included at the ratio of not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably about 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiological active peptide.

Although the emulsification ratio of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer in the sustained-release microsphere (B) may vary depending on kind of the physiologically active peptide, desired pharmacological action, duration of action and other factors, a ratio of the physiologically active peptide is usually not less than 15 wt%, preferably about 15 to about 85 wt%, more preferably about 20 to about 80 wt%, furthermore preferably about 30 to about 80 wt%, for still more preferably about 40 to about 80wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

A ratio of the pamoic acid or a salt thereof in the microsphere (B) is usually about 0.1 to about 25 wt%, preferably about 0.5 to about 15 wt%, more preferably about 1 to about 10 wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

A ratio of the biodegradable polymer in the microsphere (B) is usually about 15 to about 85 wt%, preferably about 30 to about 60 wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

In the microsphere (B) of the present invention, although the physiologically active peptides usually form salts with a pamoic acid or a salt thereof and a biodegradable polymer, a part of the physiologically active peptides may exist without forming the salts.

The particle size of the pamoic acid salt of the physiologically active peptide can be determined by observing an oil phase in the way of preparing or a cross section of the microsphere with an optical microscope, or by observing a cross section of the microsphere with an electron microscope.

The sustained-release microsphere of the present invention is of low toxicity and can be used safely to human or mammals (e.g., monkey, bovines, pigs, dogs, cats, mice, rats, rabbits, etc.) as various sustained-release preparations.

Although varying depending on kind and content of a physiologically active peptides as an active ingredient, dosage form, duration of a physiologically active peptides release, subject diseases, subject animal species, and purpose of administration, the dose of the active ingredient of the microsphere preparation may be set at any level, as long as the active ingredient is effective. For example, when the sustained-release preparation is a one-month preparation, the dose of the physiologically active peptides per administration can be chosen as appropriate over the range from about 0.001 mg to about 100 mg, preferably from about 0.01 mg to about 50 mg more preferably about 0.05 mg to about 10 mg per adult (weight 50 kg) in terms of the weight of microsphere.

More specifically, when the LH-RH antagonist represented by the general formula [Ia] above or the LH-RH agonist represented by the general formula [Ib] is used as the physiologically active peptide, it can be used as a treating or preventive agent for hormone-depending diseases such as prostatic cancer, prostatic hypertrophy, endometritis, hysteromyoma, dysmenorrhea, metrofibroma, precocious puberty, breast cancer, gallbladder cancer, cervical cancer, chronic lymphatic leukemia, chronic myelocytic leukemia, colorectal cancer, gastritis, Hodgkin's disease, malignant melanoma, metastases, multiple

myeloma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, digestive ulcers, systemic fungal infections, small cell lung cancer, valvular disease of the heart, mastopathy, polycystic ovary, infertility, chronic anovulation, appropriately induced ovulation in women, acnes, amenorrhea (e.g., secondary amenorrhea), cystic diseases of the ovary and breast (including polycystic ovary), gynecologic cancers, ovarian hyperandrogenemia and hypertrichosis, AIDS due to T-cell production mediated by thymic blastogenesis, male contraception for treatment of and male sex criminals, as an agent for contraception and mitigation of symptoms of premenstrual syndrome (PMS), as a drug for *in vitro* fertilization (IVF), and for other purposes, especially as a treating or preventive agent for prostatic cancer, prostatic hypertrophy, endometritis, hysteromyoma, metrofibroma, precocious puberty, breast cancer, etc., or an agent for contraceptive.

Although varying widely depending on dosage form, desired duration of drug release, target disease, subject animal species etc., the dose of the physiologically active peptide may be set at any level, as long as it is pharmacologically effective. The dose per administration of the drug can preferably be chosen as appropriate over the range from about 0.005 mg to about 10 mg/kg body weight per adult in the case of a 1-month sustained-release preparation. More preferably, it can be chosen as appropriate over the range from about 0.02 mg to about 5 mg/kg body weight.

The dose per administration of the microsphere in the sustained-release preparation can preferably be chosen as appropriate over the range from about 0.005 mg to 50 mg/kg body weight per adult. More preferably, it can be chosen as appropriate over the range from about 0.02 mg to 30 mg/kg body weight. Dosing frequency can be chosen as appropriate, e.g., once every several weeks, once every month, or once every several months, depending on kind and

content of active ingredient physiologically active peptide, dosage form, duration of physiologically active peptide release, target disease, subject animal species etc.

[Mode of Working the Invention]

The present invention is hereinafter described in more detail by means of the following examples, comparative examples, and experimental examples, which are not to be construed as limitative, as long as they fall within the scope of the present invention. Unless otherwise specified, % means % by weight.

Example 1

A solution of 100 mg of pamoic acid in 2.7 ml of pyridine was added to a solution of 972 mg of N-(S)-2-tetrahydrofuroyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH$_2$ (herein after abbreviated to as Compound A) acetate (produced by TAP Company) and 1040 mg of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio %) 50/50; weight-average molecular weight, 6,150; number-average molecular weight, 2,400; number-average molecular weight based on terminal group titration, 2,300; produced by Wako Pure Chemical) in 3 ml of dichloromethane. The mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (pamoic acid/Compound A (molar ratio), 0.5). After being cooled to 18°C, the suspension was poured into 400 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resultant mixture was prepared into S/O/W emulsion with the use of turbin-type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the oil phase, which was then

collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound A into the microspheres was 90.2%. Content of Compound A and molar ratio of pamoic acid/Compound A in the microspheres were 38.6% and 0.49, respectively.

Example 2

Microspheres were obtained in similar manner to Example 1, except that lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 10,100; number-average molecular weight, 3,720; number-average molecular weight based on terminal group titration, 3,500) and an amount of dichloromethane was 3.5 ml. Pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation efficiency of Compound A into the microspheres was 91.8%. Content of Compound A and pamoic acid/Compound A in the microspheres were 39.2% and 0.51, respectively.

Example 3

Microspheres were obtained in a similar manner to Example 1, except that lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 12,700; number-average molecular weight; 4,780; number-average molecular weight based on terminal group titration, 4,900) and amount of dichloromethane was 3.8 ml. Added pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation efficiency of Compound A into the microspheres was 89.9%.

Content of Compound A and pamoic acid/Compound A in the microspheres were 38.4% and 0.53, respectively.

Example 4

Microspheres were obtained in a similar manner to Example 3, except the amount of pamoic acid and pyridine in Example 1 were changed to 200 mg and 5 ml, respectively. Added pamoic acid/Compound A (molar ratio) was 1.0. Encapsulation ratio of Compound A into the microspheres was 94.1%. Content of Compound A and pamoic acid/Compound A in the microspheres were 38.3% and 0.63, respectively.

Example 5

A solution of 112 mg of disodium pamoic acid in 0.9 ml of distilled water was added to a solution of 972 mg of Compound A acetate (produced by TAP Company) and 1040 mg of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar %, 50/50); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 4 ml of dichloromethane (pamoic acid/Compound A (molar ratio), 0.5). The mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (or W/O emulsion). After being cooled to 18°C, the suspension was poured into 400 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resultant mixture was prepared into S/O/W emulsion with the use of a turbin type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal

of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound A into the microspheres was 89.8%. Content of Compound A and pamoic acid/Compound A (molar ratio) in the microspheres were 38.4% and 0.56, respectively.

Example 6

Microspheres were obtained in a similar manner to Example 5, except that the lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio %, 65/35); weight-average molecular weight, 12,500; number-average molecular weight, 4,170 and number-average molecular weight based on terminal group titration, 4,000) and the amount of dichloromethane was changed to 4.5 ml. Added pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation ratio of Compound A into the microspheres was 89.6%. Content of Compound A and pamoic acid/Compound A (molar ratio) in the microspheres were 38.3% and 0.57, respectively.

Example 7

A solution of 0.45 g of disodium pamoic acid in 3.6 ml of distilled water was added to a solution of 4.06 g of Compound A acetate (produced by TAP Company) and 4 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=50/50 (mole %); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group quantitation, 4,900; produced by Wako Pure Chemical) in 16 ml of dichloromethane (added pamoic acid/Compound A (molar ratio, 0.5)). The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (or W/O emulsion). After being cooled to 18°C, the resulting suspension was poured into 1600 ml of 0.1%

aqueous solution of polyvinylalcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previsously adjusted at 18°C. The resulting mixture was prepared into S/O/W emulsion with the use of a turbine type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. The resulting microspheres were dried at 40°C for 96 hours under reduced pressure in an oven. Encapsulation efficiency of Compound A into the obtained microspheres (average diameter 22 μm)was 93.8%. Content of Compound A and pamoic acid/Compound A (molar ratio) in the microspheres were 41.0% and 0.52, respectively.

Example 8

A solution of 0.244 g of disodium pamoic acid in 1.8 ml of distilled water was added to a solution of 2 g of NAc-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH$_2$ (herein after abbreviated to as Compound B) acetate (produced by TAP Company) and 2 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=50/50 (mole %); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 9 ml of dichloromethane (pamoic acid/Compound B (molar ratio), 0.5, content of Compound B in its acetate was supposed as 86.7 %). The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (or W/O emulsion). After

being cooled to 18°C, the suspension was poured into 800 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resulting mixture was prepared into S/O/W emulsion with the use of a turbine-type homomixer at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound B into the microspheres was 98.9%. Content of Compound B and molar ratio of pamoic acid/Compound B in the microspheres were 43.6% and 0.52, respectively.

Example 9

A solution of 0.47 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio %), 50/50); weight-average molecular weight, 18,700; number-average molecular weight, 6,180; number-average molecular weight based on terminal group titration, 6,000; produced by Wako Pure Chemical) in 15 ml of dichloromethane was added to a solution of 1,012 g of Compound A acetate (produced by TAP Company) and 0.112 g of disodium pamoic acid in 6 ml of methanol to prepare a homogeneous solution. From the solution the organic solvent was volatilized off by a rotary evaporator. The residue was sieved into particles of size of 75 μm or smaller. The resulting fine powder was again dispersed in distilled water and centrifuged at 3,000 rpm. The separated drug, etc. was

removed. The collected fine powder was again dispersed in a small amount of distilled water and lyophilized to yield powder. Encapsulation efficiency of Compound A into the powder was 94.4%. Content of Compound A and pamoic acid/Compound A (mole ratio) in the microspheres were 56.5% and 0.60, respectively.

Example 10

A solution of 0.056 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 6 ml of dichloromethane, was added to a solution of 0.506 g of Compound A acetate (produced by TAP Company) and 0.056 g of disodium pamoic acid in 3 ml of methanol to prepare a homogeneous solution. From the solution the organic solvent was volatilized off by a rotary evaporator. The residue was sieved into particles of size of 75 μm or smaller. The resulting fine powder was again dispersed in distilled water and centrifuged at 3,000 rpm. The separated drug, etc. was removed. The collected fine powder was again dispersed in a small amount of distilled water and lyophilized to yield powder. Encapsulation efficiency of Compound A in the powder was 94.6%. Content of Compound A and pamoic acid/Compound A (mole ratio) in the microspheres were 75.7% and 0.60, respectively.

Comparative Example 1

An aqueous solution of 9.4225 g of Compound A acetate was dropwisely added to a solution of 1.942 g of pamoic acid dissolved in an aqueous sodium hydroxide solution (added pamoic acid/Compound A (mole ratio)=1.0) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with

large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (mole ratio) in the lyophilized powder was 1.08.

Comparative Example 2

An aqueous solution of 54.03 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 235.5 mg of Compound A acetate (added pamoic acid/Compound A (molar ratio), 1.0) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (molar ratio) in the lyophilized powder was 1.17.

Comparative Example 3

An aqueous solution of 27.02 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 245.2 mg of Compound A acetate (added pamoic acid/Compound A (mole ratio)=0.5) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (mole ratio) in the lyophilized powder was 1.26.

Comparative Example 4

The pamoic acid salt of Compound A of Comparative Example 1 was ground. Using the salt of pamoic acid having an average particle size of 14 μm, microspheres were prepared by the following procedure.

Pamoic acid salt of Compound A (pamoic acid/Compound A (molar ratio)1.08) was added to a solution of 1.04 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio), 50/50; average-weight molecular weight,

12,700; number-average molecular weight, 4,780 and number-average molecular weight based on terminal group titration, 4,500) in 4 ml of dichloromethane. The resultant mixture was emulsified with a small homogenizer to prepare S/O suspension. Using the suspension, microspheres were prepared in a similar manner to Example 1.

Encapsulation efficiency of Compound A into the microspheres was as low as 15%. Content of Compound A was 6.1%. From the fact that pamoic acid/Compound A (mole ratio) in the microspheres was the same as 1.12 of before encapsulation, Compound A was encapsuled as the salt of pamoic acid per se and is not considered to form a salt of copolymer of lactic acid-glycolic acid.

Comparative Example 5

Microspheres were prepared in a similar manner to Example 3 except that pamoic acid and pyridine in Example 1 were not used. Encapsulation ratio of Compound A into the microspheres was 41.1%. Content of Compound A in the microspheres was 18.5%.

Comparative Example 6

An aqueous solution of 108.06 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 221.4 mg of Compound B acetate (added pamoic acid/Compound B (molar ratio, 2.0) under stirring to yield pamoic acid salt of Compound B as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, content of Compound B and pamoic acid/Compound B (mole ratio) in the lyophilized powder was 86.7% and 1.11, respectively.

Experiment 1

Microspheres produced in Examples 1-7 or pamoic acid salt of Compound A produced in Comparative Experiment 1

(sieved into particles of 25-75 μm) were used. About 6 mg of each of the particles were dispersed in 0.5 ml of a dispersant (distilled water dissolving 0.25 mg of carboxymethylcellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol). The dispersion was subcutaneously injected through 22 G needle into the back of male SD rats of 6-8 weeks. At specified intervals, rats were sacrificed, and microspheres or pamoic acid salts remained at the injected sites were collected for determination of Compound A, and the results are shown in Table 1.

Table 1

	1 week	2 weeks	3 weeks	4 weeks
Example 1	46%	22%	14%	10%
Example 2	56%	27%	16%	13%
Example 3	63%	35%	19%	12%
Example 4	51%	35%	20%	15%
Example 5	63%	33%	16%	9%
Example 6	63%	36%	21%	12%
Example 7	67%	28%	22%	5%
Ref.Ex. 1	40%	18%	13%	4%

Example 11

An aqueous solution of 500 mg of 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 acetate (produced by Takeda Chemical Industries, Ltd. Herein after abbreviated to as Compound C) in 0.45 ml of distilled water was added to a solution of 1,800 mg of polylactic acid (average-weight molecular weight, 50,000; number-average molecular weight, 25,000; produced by Taki Chemical) in 7.5 ml of dichloromethane. The resulting mixture was emulsified using a small homogenizer for 60 seconds to produce W/O emulsion, followed by addition of 0.85 ml of a solution of 85 mg of disodium pamoic acid dissolved in methanol. The resulting mixture was again emulsified using a small homogenizer for 60 seconds to yield S/O suspension. After

being cooled to 18°C, the suspension was poured into 400 ml of 0.1 % aqueous solution of polyvinyl alcohol (EG-40, Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% of mannitol. Using a turbine-type homomixer, the mixture was prepared into S/O/W emulsion at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off dichloromethane and to solidify the oil phase, which was collected by centrifuge (5PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting microspheres were dispersed in distilled water and further centrfiuged, followed by removing the separated drug, etc.. The collected microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound C into the microspheres was 86.4%. Content of Compound C and pamoic acid/Compound A (mole ratio) in the microspheres were 18.2% and 0.50, respectively.

Example 12

Microspheres were prepared in a similar manner to Example 11, except that the polylactic acid was replaced by 1,500 mg of polylactic acid (weight-average molecular weight, 17,000; number-average molecular weight, 5,000; number-average molecular weight based on terminal group titration, 5,500; produced by Wako Pure Chemical), the amount of dichloromethane was changed to 8 ml and the methanol solution of disodium pamoic acid was replaced by 1.1 ml of a distilled water solution. Encapsulation efficiency of Compound C into the microspheres was 92.8%. Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 21.9% and 0.78, respectively.

Example 13

An aqueous solution of 1,000 mg of Compound C dissolved in 0.9 ml of distilled water was added to a

solution of 3,600 mg of lactic acid (weight-average molecular weight, 24.300; number-average molecular weight, 7.790; number-average molecular weight based on terminal group titration, 8,000; produced by Wako Pure Chemical) in 8 ml of dichloromethane. The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield W/O emulsion, followed by addition of a solution of 204 ml of disodium pamoic acid in 2 ml of methanol. The resulting mixture was again emulsified using a small homogenizer for 60 seconds to produce an almost clear but slightly opaque yellow solution. After being cooled to 18°C, the resulting yellow solution was poured into 800 ml of an aqueous solution of 0.1% polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical) containing 5% mannitol. The resulting mixture was prepared into O/W emulsion by a turbine-type homomixer at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was collected by centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting microspheres were again dispersed in distilled water and centrifuged, followed by removing the separated drug, etc.. The collected microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound C into the microspheres (average diameter 25 μm) was 100.0%. Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 20.9% and 0.57, respectively.

Example 14

Microspheres were prepared in a similar manner to Example 13 using yellow solution as prepared in Example 13, except that the polylactic acid was replaced by one (weight-average molecular weight, 40,000; number-average molecular weight, 26,700; produced by Taki Chemical) and

the amount of dichloromethane was changed to 9 ml. Encapsulation efficiency of Compound C into the microspheres was 100.3%. Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 21.0% and 0.56, respectively.

Example 15

Microspheres were prepared in a similar manner to Example 13 except that amounts of the polylactic acid, dichloromethane, disodium pamoic acid and methanol were changed to 5000 mg, 10 ml, 130 mg and 1.3 ml, respectively (the oil phase was not a solution observed in Example 13 but a W/O emulsion). The resulting microspheres were further dried at 55°C for 120 hours under reduced pressure in an oven. Encapsulation efficiency of Compound C into the microspheres was 100.0%. Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 16.4% and 0.39, respectively.

Comparative Example 7

Microspheres were prepared in a similar manner to Example 11, except that the methanol solution of disodium pamoic acid was not added. Content of Compound C in the obtained microspheres was 7.7%.

Comparative Example 8

An aqueous solution of 500 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 2936.5 mg of Compound C (added pamoic acid/Compound C (molar ratio), 0.5) under stirring to yield pamoic acid salt. The resulting salt was washed with large excess of water and lyophilized. By HPLC determination, pamoic acid/Compound C (molar ratio) in the lyophilized powder was 0.87. The powder was sieved to obtain particles of an average particle size of 10 μm. The resulting powder were added to a solution of 1,800 mg of polyactic acid in 7.5 ml of

dichloromethane so that an amount of Compound C is equal to that in Example 11. Thus, microspheres were prepared using oil phase, wherein pamoic acid salt of Compound C was dispersed, by in-water drying method as a similar manner to Example 11, except that neither the aqueous solution of Compound C nor methanol solution of disodium pamoic aicd was added. Content of Compound C in the obtained microspheres was 7.4%.

Comparative Example 9

Microspheres were prepared in a similar manner to Example 12, except that an aqueous solution of disodium pamoic acid in distilled water was not added. Content of Compound C in the obtained microspheres was 11.3%.

INDUSTRIAL APPLICABILITY

The microsphere of the present invention contains a large amount of the physiologically active peptide and can regulate a release rate of the physiologically peptide.

CLAIMS

1. A method of producing a sustained-release microsphere which comprises emulsification of a physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and a pamoic acid or an alkaline metal salt thereof with a biodegradable polymer.

2. The method according to claim 1, which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and a solution of the pamoic acid or an alkaline metal salt thereof in a solution of the biodegradable polymer with an organic solvent, and removing the solvent.

3. The method according to claim 1, which comprises dissolving the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt, the pamoic acid or an alkaline metal salt thereof and the biodegradable polymer in an organic solvent, and removing the solvent.

4. The method according to claim 1, which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and the biodegradable polymer with an organic solvent and a solution of the pamoic acid or an alkaline metal salt thereof, and removing the solvent.

5. The method according to claim 1, which comprises emulsification of a solution of the biodegradable polymer and the pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt, and removing the solvent.

6. The method according to any one of claims 2 to 5, wherein the removing of the solvent is conducted by in-water drying method.

7. The method according to claim 6, followed by freeze drying.

8. The method according to any one of claims 2 to 5, wherein a concentration of the physiologically active peptide in the solution mixture is about 1 to about 25 wt% of the solution mixture.

9. The method according to any one of claims 2 to 5, wherein a concentration of the biodegradable polymer in the solution mixture is about 1 to about 25 wt% of the solution mixture.

10. The method according to any one of claims 2 to 5, wherein a concentration of the pamoic acid or a salt thereof in the solution mixture is about 0.05 to about 5 wt% of the solution mixture.

11. The method according to claim 2 or 4, wherein the solution of the pamoic acid or a salt thereof is a methanol solution of the pamoic acid or a salt thereof.

12. The method according to claim 4, wherein an amount of the solution of the pamoic acid or a salt thereof is about 2 to about 90 (v/v) % to the organic solvent of the physiologically active peptide and the biodegradable polymer in the solution mixture.

13. The method according to claim 1, wherein the physiologically active peptide or a salt thereof is a free base or a salt with a weak acid of not less than pKa 4.0.

14. The method according to claim 1, wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid.

15. The method according to claim 1, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid.

16. The method according to claim 1, wherein the physiologically active peptide is an LH-RH agonist.

17. The method according to claim 1, wherein the physiologically active peptide is an LH-RH antagonist.

18. The method according to claim 1, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof.

19. The method according to claim 1, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 acetate.

20. The method according to claim 1, wherein the biodegradable polymer is a polymer of α-hydroxy carboxylic acids.

21. The method according to claim 20, wherein the polymer of α-hydroxy carboxylic acids is a lactic acid/glycolic acid polymer.

22. The method according to claim 21, wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%).

23. The method according to claim 20, wherein a weight-average molecular weight of the biodegradable polymer is 3,000 to 100,000.

24. The method according to claim 1, wherein the biodegradable polymer is a polylactic acid.

25. The method according to claim 24, wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000.

26. The method according to any one of claims 2 to 5, wherein the organic solvent is a dichloromethane.

27. The method according to claim 1, wherein the physiologically active peptide is a peptide having one basic group capable of forming a salt with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising an about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide.

28. The method according to claim 1, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid, and the sustained-release microsphere is a sustained-

release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

29. A sustained-release microsphere which is obtainable by the method according to claim 1.

30. A sustained-release microsphere which comprises an about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide and a biodegradable polymer.

31. A sustained-release microsphere which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

32. A sustained-release microsphere which comprises not more than about 0.8 mol of pamoic acid to 1 mol of physiologically active peptide.

33. The sustained-release microsphere according to claim 32, which comprises about 0.3 to about 0.7 mol of the pamoic acid to 1 mol of the physiologically active peptide.

34. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a physiologically active peptide having basic groups capable of forming salts with a weak acid of not less than pKa4.0.

35. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid.

36. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid.

37. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is an LH-RH agonist.

38. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is an LH-RH antagonist.

39. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof.

40. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 acetate.

41. The sustained-release microsphere according to claim 29 or 31, wherein the biodegradable polymer is a polymer of α-hydroxy carboxylic acids.

42. The sustained-release microsphere according to claim 41, wherein the polymer of α-hydroxy carboxylic acids is a lactic acid/glycolic acid polymer.

43. The sustained-release microsphere according to claim 42, wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%).

44. The sustained-release microsphere according to claim 41, wherein a weight-average molecular weight of the polymer is 3,000 to 100,000.

45. The sustained-release microsphere according to any one of claim 29 to 32, wherein the biodegradable polymer is a polylactic acid.

46. The sustained-release microsphere according to claim 45, wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000.

47. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the physiologically active peptide in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere.

48. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the pamoic acid

or a salt thereof in the sustained-release microsphere is about 0.1 to about 25 wt% of the sustained-release microsphere.

49. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the biodegradable polymer in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere.

50. The sustained-release microsphere according to claim 30, wherein a ratio of the about 0.01 to about 10 μm particle size of a pamoic acid salt of the physiologically active peptide in the sustained-release microsphere is about 15 to about 90 wt% of the sustained-release microsphere.

51 The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C_2H_5 or a salt thereof and a content of the peptide is about 15 to about 30 wt% to the total microcapsule.

52. A sustained-release microsphere which is produced by the method according to claim 1.

53. A sustained-release preparation which comprises the microsphere according to any one of claims 29 to 32.

54. The sustained-release preparation according to claim 53, which is an injectable preparation.

55. A sustained-release preparation which comprises the microsphere according to claim 37 or 38.

56. The sustained-release preparation according to claim 55, which is a treating or preventive agent for prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma, dysmenorrhea, precocious puberty or breast cancer, or a contraceptive agent.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/JP 98/00339

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/16 A61K9/50 A61K47/12 A61K38/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	GB 2 257 909 A (DEBIO RECH PHARMA SA) 27 January 1993 cited in the application see the whole document ---	29-31, 34-36, 41-56
X,Y	EP 0 626 170 A (SANDOZ AG ;SANDOZ LTD (CH); SANDOZ AG (DE)) 30 November 1994 see the whole document ---	29-31, 34-36, 41-56
Y	DE 38 22 459 A (BPD BIOPHARM DEV LTD) 30 March 1989 see the whole document ---	29-31, 34-36, 41-56
A	GB 2 234 169 A (DEBIOPHARM SA) 30 January 1991 see the whole document ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1998	08/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fischer, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/JP 98/00339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
A	WO 95 15767 A (ASTA MEDICA AG ;ENGEL JUERGEN (DE); KLOKKERS BETHKE KARIN (DE); RE) 15 June 1995 cited in the application see the whole document	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/JP 98/00339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2257909 A	27-01-93	CH 683149 A	31-01-94
		AT 403348 B	26-01-98
		AT 148992 A	15-06-97
		AU 651711 B	28-07-94
		AU 2043692 A	28-01-93
		AU 652844 B	08-09-94
		AU 2043792 A	28-01-93
		BE 1005696 A	21-12-93
		BE 1005697 A	21-12-93
		BR 9205375 A	08-03-94
		CA 2074320 A	23-01-93
		CA 2074322 A	23-01-93
		CH 683592 A	15-04-94
		WO 9301802 A	04-02-93
		CN 1070344 A	31-03-93
		CZ 9300660 A	19-01-94
		DE 4223282 A	28-01-93
		DE 4223284 A	28-01-93
		DE 9219084 U	25-09-97
		DK 93892 A	23-01-93
		DK 93992 A	23-01-93
		ES 2037621 B	01-02-94
		ES 2050070 A	01-05-94
		FI 923320 A	23-01-93
		FI 923321 A	23-01-93
		FR 2679450 A	29-01-93
		FR 2680109 A	12-02-93
		GB 2257973 A,B	27-01-93
		GR 1001446 B	30-12-93
		GR 92100323 A	24-05-93
		HR 920229 A	30-04-96
		HU 64234 A	28-12-93
		IE 69967 B	16-10-96
		IE 71199 B	12-02-97
		IL 102590 A	13-07-97
		IL 102591 A	10-06-97
		IT 1259891 B	28-03-96
		IT 1259892 B	28-03-96
		JP 6172208 A	21-06-94
		JP 5221855 A	31-08-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/JP 98/00339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2257909 A		LU 88150 A	15-02-93
		LU 88151 A	15-02-93
		MX 9204268 A	31-03-94
		NL 9201309 A	16-02-93
		NL 9201310 A	16-02-93
		PL 298504 A	10-01-94
		PT 100712 A	29-10-93
		PT 100713 A	31-08-93
		SE 9202212 A	23-01-93
		SE 9202213 A	23-01-93
EP 0626170 A	30-11-94	CA 2123144 A	11-11-94
		JP 6340543 A	13-12-94
DE 3822459 A	30-03-89	GB 2209937 A	01-06-89
		AT 397035 B	25-01-94
		AU 2232688 A	23-03-89
		BE 1001685 A	06-02-90
		CA 1326438 A	25-01-94
		CH 675968 A	30-11-90
		DK 518988 A	22-03-89
		FI 884297 A,B,	22-03-89
		FR 2620621 A	24-03-89
		GR 88100619 A	22-06-89
		IE 60608 B	27-07-94
		JP 1121222 A	12-05-89
		JP 1981991 C	25-10-95
		JP 7013023 B	15-02-95
		LU 87340 A	06-04-89
		NL 8802323 A	17-04-89
		NO 178604 B	22-01-96
		PT 88557 A,B	01-10-88
		SE 503406 C	10-06-96
		SE 8803321 A	22-03-89
		US 5192741 A	09-03-93
GB 2234169 A	30-01-91	CH 679207 A	15-01-92
		AT 397197 B	25-02-94
		AU 619996 B	06-02-92
		AU 5910390 A	31-01-91

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/JP 98/00339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2234169 A		BE 1003093 A	19-11-91
		CA 2021767 A,C	29-01-91
		DE 4023134 A	31-01-91
		DK 180790 A	29-01-91
		FI 97688 B	31-10-96
		FR 2650182 A	01-02-91
		GR 1001215 B	21-06-93
		IE 65397 B	18-10-95
		IL 95120 A	07-10-94
		IT 1243357 B	10-06-94
		JP 1933192 C	26-05-95
		JP 3066625 A	22-03-91
		JP 6062427 B	17-08-94
		LU 87772 A	11-12-90
		NL 9001646 A	18-02-91
		NO 300304 B	12-05-97
		PT 94842 A,B	20-03-91
		SE 504279 C	23-12-96
		SE 9002522 A	29-01-91
		US 5134122 A	28-07-92
		US 5439688 A	08-08-95
		US 5225205 A	06-07-93
WO 9515767 A	15-06-95	DE 4342092 A	14-06-95
		AU 677748 B	01-05-97
		AU 1219995 A	27-06-95
		BR 9408272 A	17-12-96
		CA 2178592 A	15-06-95
		CN 1136779 A	27-11-96
		CZ 9601420 A	11-12-96
		EP 0732934 A	25-09-96
		FI 962354 A	06-06-96
		HR 940978 A	28-02-97
		HU 75712 A	28-05-97
		JP 9509145 T	16-09-97
		LV 11596 B	20-06-97
		NO 961877 A	09-05-96
		NZ 277239 A	24-03-97
		PL 314913 A	30-09-96
		SK 66796 A	06-08-97

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/JP 98/00339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9515767 A		ZA 9409798 A	18-08-95

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/09, 9/16, 9/50 | A1

(11) International Publication Number: **WO 96/22786**

(43) International Publication Date: 1 August 1996 (01.08.96)

(21) International Application Number: PCT/JP96/00090

(22) International Filing Date: 19 January 1996 (19.01.96)

(30) Priority Data:
7/8224 23 January 1995 (23.01.95) JP

(71) Applicant *(for all designated States except US)*: TAKEDA CHEMICAL INDUSTRIES, LTD. [JP/JP]; 1-1, Doshomachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541 (JP).

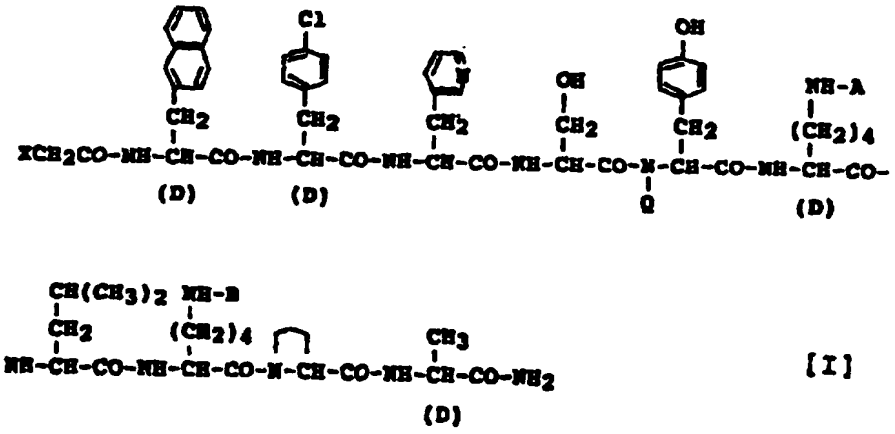
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: IGARI, Yasutaka [JP/JP]; 4-25-503, Motoyamaminamimachi 5-chome, Higashinada-ku, Kobe-shi, Hyogo 658 (JP). SAIKAWA, Akira [JP/JP]; 50-1, Yamadaminami, Suita-shi, Osaka 565 (JP). KAMEI, Shigeru [JP/JP]; 7-1-509, Sumiregaoka 1-chome, Takarazuka-shi, Hyogo 665 (JP).

(74) Agents: ASAHINA, Tadao et al.; Osaka Plant of Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532 (JP).

(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SUSTAINED-RELEASE PREPARATION AND USE



[I]

(57) Abstract

This invention provides a sustained-release preparation production method comprising production of a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by formula (I), wherein X represents a hydrogen atom or a tetrahydrofurylcarboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion. According to the production method of the present invention, a sustained-release preparation containing peptide (I) or a salt thereof can be obtained easily and at high recovery rates.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

DESCRIPTION

SUSTAINED-RELEASE PREPARATION AND USE

Technical Field

The present invention relates to a method of producing a sustained-release preparation containing a bioactive peptide possessing LH-RH-antagonizing activity or a salt thereof.

Background Art

As a prior art method, EP-A-601,799, for instance, describes a method of producing a sustained-release preparation (in-water drying method using an O/W emulsion, phase separation method and spray drying method), by dissolving both a bioactive peptide and a biodegradable polymer having a free carboxyl group at one end in a substantially water-immiscible solvent, then removing the solvent.

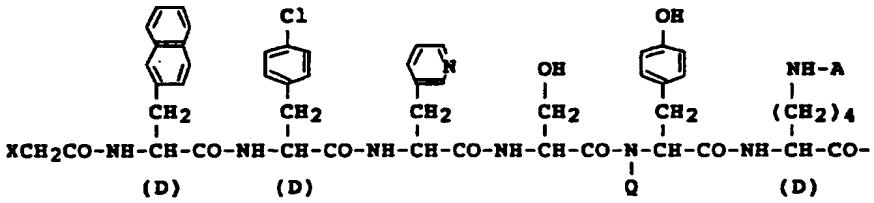
Disclosure of Invention

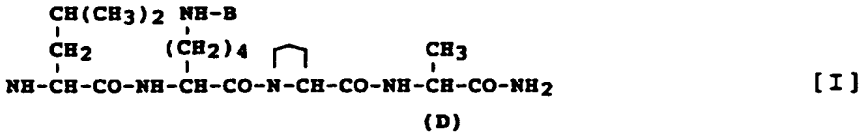
Although use of first- or second-generation LH-RH (lutein-izing hormone-releasing hormone) antagonists has been problematic because of their histamine-releasing action (Gekkan Yakuji, Vol. 32, pp. 1599-1605, 1990), a large number of compounds have been synthesized, resulting in the recent development of LH-RH-antagonizing bioactive peptides without the problem of histamine-releasing action (e.g., Japanese Patent Unexamined Publication No. 101695/1991). For such LH-RH-antagonizing bioactive peptides to exhibit pharmaceutical effect, they must competitively inhibit LH-RH action constantly in the body. Accordingly, there is need for the development of sustained-release preparations such peptides. In addition, there is also need for the development of a method of producing a sustained-release preparation in which excess

drug release is suppressed just after administration, since such bioactive peptides possess low but not negligible histamine-releasing activity. Also, in sustained-release preparations of the long-acting type (e.g., 1-3 months), more reliable, constant release of bioactive peptide is a key to safe and more reliable effect. There is need for a method of producing a sustained-release preparation that constantly releases a bioactive peptide and that possesses excellent storage stability.

The present invention relates to:

(1) a method of producing a sustained-release preparation, which comprises producing a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by the formula:



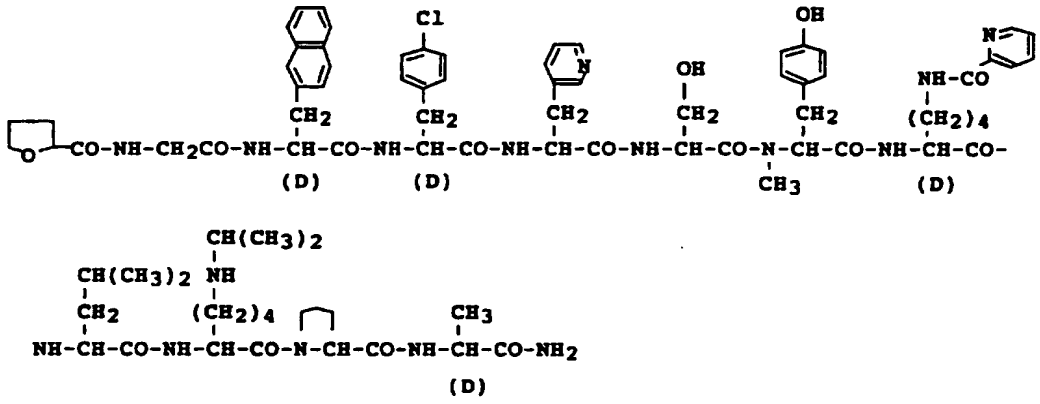


[I]

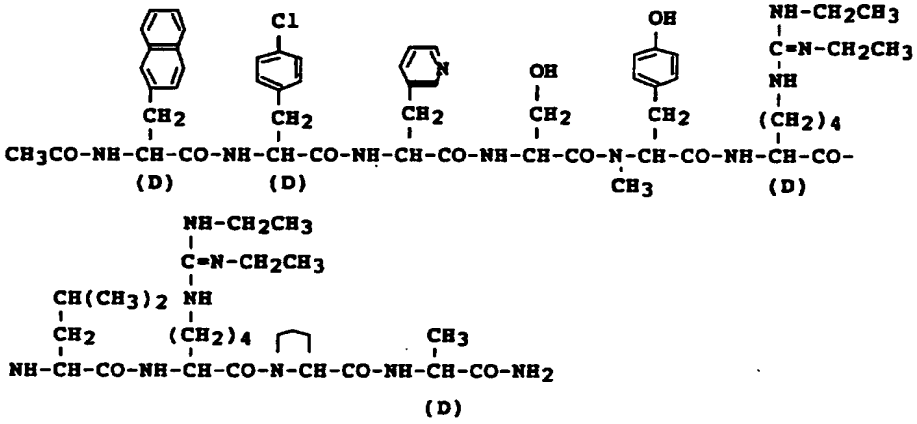
wherein X represents a hydrogen atom or a tetrahydrofuryl-carboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion,

(2) a method of term 1 above, wherein the biodegradable polymer is an aliphatic polyester,

(3) a method of term 2 above, wherein the aliphatic polyester is a lactic acid-glycolic acid copolymer,
(4) a method of term 3 above, wherein a composition ratio of lactic acid and glycolic acid is about 100/0 to about 40/60 (mole%),
(5) a method of term 3 above, wherein a weight-average molecular weight of the copolymer is about 5,000 to about 25,000,
(6) a method of term 1 above, wherein a peptide concentration in the internal aqueous phase is about 0.1 to about 150% (w/v),
(7) a method of term 1 above, wherein a polymer concentration in the oil phase is about 0.01 to about 80% (w/w),
(8) a method of term 1 above, wherein a volume ratio of the internal aqueous and oil phase is about 1 to about 50% (v/v),
(9) a method of term 1 above, wherein a volume of the external aqueous phase is about 1 to about 10,000 times that of the oil phase,
(10) a method of term 1 above, wherein the preparation is microcapsules,
(11) a method of term 1 above, wherein X is 2-tetrahydrofurylcarboxamido,
(12) a method of term 11, wherein the 2-tetrahydrofurylcarboxamido is (2S)-tetrahydrofurylcarboxamido,
(13) a method of term 1 above, wherein the peptide is of the formula:



(14) a method of term 1 above, wherein the peptide is of the formula:



(15) a sustained-release preparation, which is produced by the method of term 1 above,

(16) a preparation of term 15 above, wherein a content ratio of the peptide is about 0.01 to about 50% (w/w), relative to the polymer,

(17) a preparation of term 15 above, wherein the preparation is microcapsules, and

(18) a preparation of term 17 above, wherein the microcapsules are for injection.

Abbreviations used in the present specification have the following meanings.

NAcD2Nal : N-acetyl-D-3-(2-naphthyl)alanyl
D4ClPhe : D-3-(4-chlorophenyl)alanyl
D3Pal : D-3-(3-pyridyl)alanyl
NMeTyr : N-methyltyrosyl
DLys(Nic) : D-(ipsiron-N-nicotinoyl)lysyl
Lys(Nisp) : (Ipsiron-N-isopropyl)lysyl
DhArg(Et_2): D-(N,N'-diethyl)homoarginyl

Abbreviations for other amino acids are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature (European Journal of Biochemistry, Vol. 138, pp. 9-37, 1984) or abbreviations in common use in relevant fields. When an optical isomer may be present in amino acid, it is of the L-configuration, unless otherwise stated.

In the present invention, the bioactive peptide represented by formula [I] (hereinafter also referred to as peptide [I]) or a salt thereof possesses LH-RH-antagonizing activity, and accordingly, is effective in the treatment of hormone-dependent diseases, such as prostatic cancer, prostatic hypertrophy, endometriosis, uterine myoma, uterine fibroma, precocious puberty, breast cancer, bladder cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, gastritis, hodgkin's disease, malignant melanoma, metastasis, multiple myeloma, non-hodgkin's leukemia, non-small-cell lung cancer, ovarian cancer, peptic ulcer, serious fungal infection, small-cell lung cancer, valvular heart disease, mastopathy, polycystic ovary, infertility, controlled induction of ovulation in women with chronic anovulation, acne, amenorrhea (e.g., secondary amenorrhea), ovarian and mammary cystic disease (including, polycystic ovarian diseases), gynecological cancer, ovarian hyperandrogenism and hirsutism, AIDS by rejuvenating the thymus to produce T-cells, male contraceptives for the treatment of male sex

offenders and in contraception, symptomatic relief of the premenstrual syndrome (PMS), in vitro fertilization.

With respect to the formula [I], X is preferably 2-tetrahydrofurylcarboxamido, more preferably (2S)-tetrahydrofurylcarboxamido. Also, A is preferably nicotinoyl; B is preferably isopropyl.

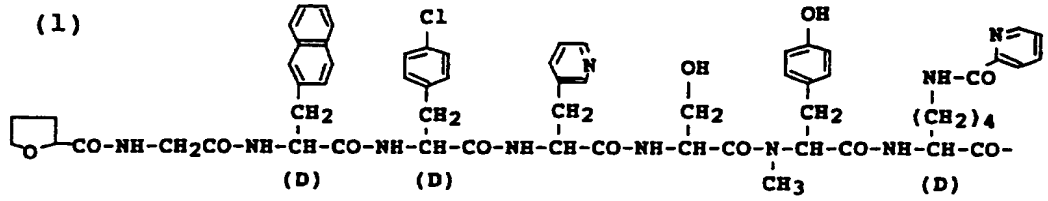
When peptide [I] has one or more kinds of asymmetric carbon atoms, two or more optical isomers are present. Such optical isomers and mixtures thereof are also included in the scope of the present invention.

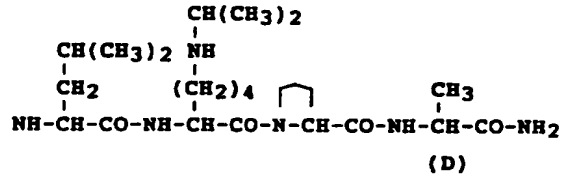
Peptide [I] or a salt thereof can be produced by known methods, which include those methods described in Japanese Patent Unexamined Publication No. 101695/1994 and the Journal of Medicinal Chemistry, Vol. 35, p. 3942 (1992) and other publications, and similar methods.

The salt of peptide [I] is preferably a pharmacologically acceptable salt. Such salts include salts with inorganic acids (e.g., hydrochloric acid, sulfuric acid, nitric acid, etc.), organic acids (e.g., carbonic acid, bicar-bonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid, etc.) etc. More preferably, the salt of peptide [I] is a salt with an organic acid (e.g., carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid, etc.), with greater preference given to the salt with acetic acid. These salts may be mono- to tri-salts, with preference given to di- or tri-salts.

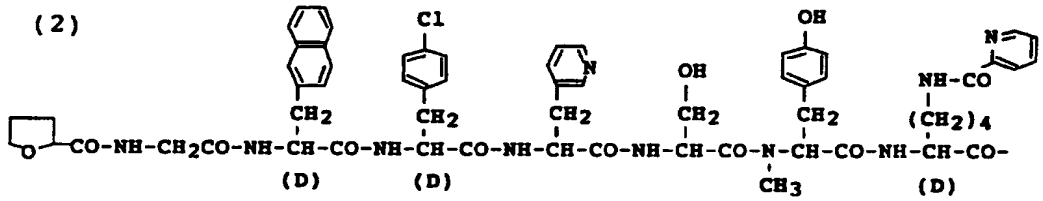
Examples of particularly preferable peptide [I] compounds or salts thereof are given below.

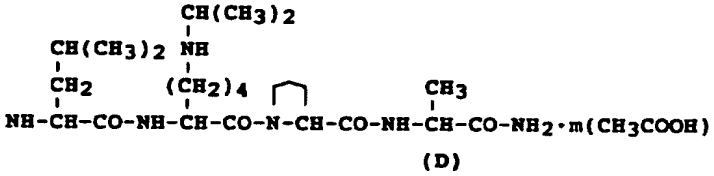
(1)





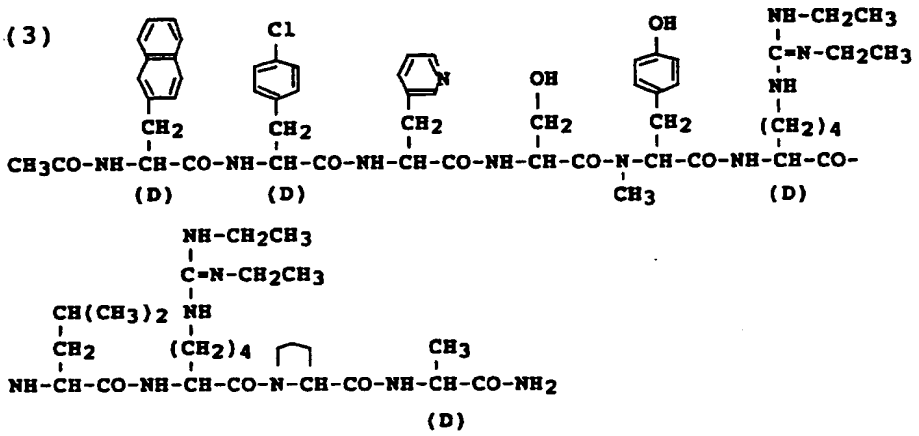
(2)



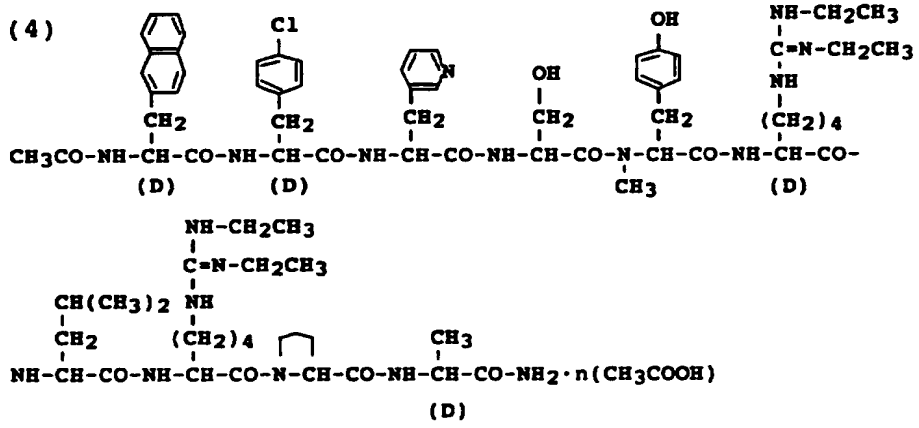


wherein, m represents an integer from 1 to 3.

(3)



(4)



wherein, n represents an integer from 1 to 3.

Peptide [I] or salt thereof is preferably (1) or (2) above.

The biodegradable polymer having a free carboxyl group at one end is a biodegradable polymer whose GPC measurement- and terminal group quantitation-based number-average molecular weights almost agree with each other.

Number-average molecular weight based on terminal group quantitation is calculated as follows:

About 1 to 3 g of the biodegradable polymer is dissolved in a mixed solvent of acetone (25 ml) and

methanol (5 ml); the solution is quickly titrated with a 0.05 N alcoholic solution of potassium hydroxide while stirring at room temperature (20°C), with phenolphthalein as an indicator to determine carboxyl group content; the number-average molecular weight is calculated from the following equation:

Number-average molecular weight based on terminal group quantitation = 20,000 × A/B

where A is the weight mass (g) of the biodegradable polymer, and B is the amount (ml) of the 0.05 N alcoholic solution of potassium hydroxide added until the titration end point is reached.

For example, in the case of a polymer having a free carboxyl group at one end, and synthesized from one or more α-hydroxy acids by catalyst-free dehydration polymerization condensation, the GPC measurement- and terminal group quantitation-based number-average molecular weights almost agree with each other. On the other hand, in the case of a polymer having substantially no free carboxyl group at one end, and synthesized from a cyclic dimer by ring-opening polymerization using a catalyst, the number-average molecular weight based on terminal group quantitation is significantly higher than that based on GPC measurement. This difference makes it possible to clearly differentiate a polymer having a free carboxyl group at one end from a polymer having substantially no free carboxyl group at one end.

While the number-average molecular weight based on terminal group quantitation is an absolute value, that based on GPC measurement is a relative value, that varies depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width, baseline). It is therefore difficult to have an absolute numerical representation of both values. However, the fact that the GPC measurement- and terminal group

quantitation-based number-average molecular weights almost agree with each other means that the number-average molecular weight based on terminal group quantitation falls within the range from about 0.4 to about 2 times, preferably from about 0.5 to about 2 times, and more preferably from about 0.8 to about 1.5 times, that based on GPC measurement. Also, the fact that the number-average molecular weight based on terminal group quantitation is significantly higher than that based on GPC measurement means that the number-average molecular weight based on terminal group quantitation is about 2 times or more that based on GPC measurement.

Examples of biodegradable polymers having a free carboxyl group at one end include polymers, copolymers, or mixtures thereof, synthesized by catalyst-free dehydration polymerization condensation from one or more α-hydroxycarboxylic acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid, etc.), hydroxydicarboxylic acids (e.g., malic acid, etc.), hydroxytricarboxylic acids (e.g., citric acid, etc.) etc., poly-α-cyanoacrylates, polyamino acids (e.g., poly-γ-benzyl-L-glutamic acid, etc.), maleic anhydride copolymers (e.g., styrene-maleic acid copolymer, etc.) and the like.

The biodegradable polymer is preferably an aliphatic polyester such as a homopolymer, copolymer or mixture thereof synthesized from one or more α-hydroxycarboxylic acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid, etc.), hydroxydicarboxylic acids (e.g., malic acid, etc.), hydroxytricarboxylic acids (e.g., citric acid, etc.) and so on.

Polymerization may be of the random, block or graft type. When the above-mentioned α-hydroxy acids, hydroxydicarboxylic acids and hydroxytricarboxylic acids have an optically active center in their molecular structures, they may be of the D-, L- or DL-configuration.

The biodegradable polymer having a free carboxyl group at one end is preferably (1) a lactic acid-glycolic acid copolymer or (2) a biodegradable polymer comprising a mixture of (A) a copolymer of glycolic acid and a hydroxycarboxylic acid represented by the formula:

[II]

wherein R represents an alkyl group having 2 to 8 carbon atoms, and (B) polylactic acid. More preferably, the biodegradable polymer having a free carboxyl group at one end is a lactic acid-glycolic acid copolymer.

When a lactic acid/glycolic acid copolymer is used as the biodegradable polymer, its content ratio (lactic acid/glycolic acid) (mol%) is preferably about 100/0 to about 40/60, more preferably about 90/10 to about 50/50.

The weight-average molecular weight of the lactic acid/glycolic acid copolymer is preferably about 5,000 to about 25,000, more preferably about 7,000 to about 20,000, still more preferably about 8,000 to about 15,000.

The degree of dispersion of the lactic acid/glycolic acid copolymer (weight-average molecular weight/number-average molecular weight) is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The lactic acid-glycolic acid copolymer can be produced by a known production method, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

The decomposition/elimination rate of a lactic acid/glycolic acid copolymer varies widely, depending on composition or molecular weight. However, drug release duration can be extended by lowering the glycolic acid

ratio or increasing the molecular weight, since decomposition/elimination is delayed as the glycolic acid ratio decreases. Conversely, drug release duration can be shortened by increasing the glycolic acid ratio or decreasing the molecular weight. To obtain a sustained-release preparation of the long acting type (e.g., 1-4 months), it is preferable to use a lactic acid-glycolic acid copolymer whose content ratio and weight-average molecular weight fall in the above ranges. If choosing a lactic acid-glycolic acid copolymer that decomposes more rapidly than that whose content ratio and weight-average molecular weight fall in the above ranges, the initial burst is difficult to suppress; if choosing a lactic acid-glycolic acid copolymer that decomposes more slowly than that whose content ratio and weight-average molecular weight fall in the above ranges, it is likely that no effective amount of drug is released for a certain period of time.

With respect to the formula [II] above, the linear or branched alkyl group represented by R, which has 2 to 8 carbon atoms, is exemplified by ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, tert-pentyl, 1-ethylpropyl, hexyl, isohexyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl and 2-ethylbutyl. Preferably, a linear or branched alkyl group having 2 to 5 carbon atoms is used. Such alkyl groups include ethyl, propyl, isopropyl, butyl and isobutyl. More preferably, R is ethyl.

The hydroxycarboxylic acid represented by the formula [II] is exemplified by 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid, 2-hydroxyisocaproic acid and 2-hydroxycapric acid, with preference given to 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methyl-

butyric acid and 2-hydroxycaproic acid, with greater preference given to 2-hydroxybutyric acid. Although the hydroxycarboxylic acid may be of the D-, L- or D,L-configuration, it is preferable to use a mixture of the D- and L-configurations wherein the ratio of the D-/L-configuration (mol%) preferably falls within the range from about 75/25 to about 25/75, more preferably from about 60/40 to about 40/60, and still more preferably from about 55/45 to about 45/55.

With respect to the copolymer of glycolic acid and the hydroxycarboxylic acid represented by the formula [II] (hereinafter glycolic acid copolymer (A)), polymerization may be of random, block or graft type. A random copolymer is preferred.

The hydroxycarboxylic acid represented by the formula [II] may be a mixture of one or more kinds in a given ratio.

With respect to the content ratio of glycolic acid and the hydroxycarboxylic acid represented by the formula [II] in glycolic acid copolymer (A), it is preferable that glycolic acid account for about 10 to 75 mol% and hydroxycarboxylic acid for the remaining portion. More preferably, glycolic acid accounts for about 20 to about 75 mol%, and still more preferably about 40 to about 70 mol%. The weight-average molecular weight of the glycolic acid copolymer is normally about 2,000 to about 50,000, preferably about 3,000 to about 40,000, and more preferably about 8,000 to about 30,000. The degree of dispersion of the glycolic acid copolymer (weight-average molecular weight/number-average molecular weight) is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

Glycolic acid copolymer (A) above can be produced by a known processes, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

Although the polylactic acid may also be of the D- or L-configuration or a mixture thereof, it is preferable that the ratio of the D-/L-configuration (mol%) falls within the range from about 75/25 to about 20/80. The ratio of the D-/L-configuration (mol%) is more preferably about 60/40 to about 25/75, and still more preferably about 55/45 to about 25/75. The weight-average molecular weight of the polylactic acid is preferably about 1,500 to about 30,000, more preferably about 2,000 to about 20,000, and still more preferably about 3,000 to about 15,000. Also, the degree of dispersion of the polylactic acid is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

For producing polylactic acid, two methods are known: ring-opening polymerization of lactide, a dimer of lactic acid, and dehydration polymerization condensation of lactic acid. For obtaining a polylactic acid of relatively low molecular weight for the present invention, direct dehydration polymerization condensation of lactic acid is preferred. This method is, for example, described in Japanese Patent Unexamined Publication No. 28521/1986.

Glycolic acid copolymer (A) and polylactic acid (B) are used in a mixture wherein the (A)/(B) ratio (% by weight) falls within the range from about 10/90 to about 90/10. The mixing ratio is preferably about 20/80 to about 80/20, and more preferably about 30/70 to about 70/30. If either component (A) or (B) is in excess, the preparation obtained shows a drug release pattern no more than that obtained with the use of component (A) or (B) alone; no linear release pattern is expected in the last stage of drug release from the mixed base. Although the decomposition/elimination rates of glycolic acid copolymer (A) and polylactic acid vary widely, depending on molecular weight or composition, drug release duration can be extended by increasing the molecular weight of the polylactic acid or lowering the mixing ratio (A)/(B), since the decomposi-

tion/elimination rate of glycolic acid copolymer (A) is usually higher than that of polylactic acid. Conversely, drug release duration can be shortened by decreasing the molecular weight of polylactic acid or increasing the mixing ratio (A)/(B). Drug release duration can also be adjusted by altering the kind and content ratio of hydroxycarboxylic acid represented by the formula [II].

In the present specification, weight-average molecular weight and degree of dispersion are defined as the molecular weight based on polystyrene obtained by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with respective weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, and degree of dispersion calculated. Measurements were taken using a GPC column KF804L×2 (produced by Showa Denko, Japan) and an RI monitor L-3300 (produced by Hitachi, Ltd., Japan) with chloroform as the mobile phase.

The production method of the present invention is hereinafter described in detail.

First, peptide [I] or a salt thereof (hereinafter also referred to as a drug) is dissolved or dispersed in water, with a drug support when necessary, such as gelatin, agar, polyvinyl alcohol or a basic amino acid (e.g., arginine, histidine, lysine), dissolved or suspended, to yield an internal aqueous phase.

The drug concentration in the internal aqueous phase is preferably about 0.1 to about 150% (w/v), more preferably about 20 to about 130% (w/v), and still more preferably about 60 to about 120% (w/v).

The internal aqueous phase also may be supplemented with a pH regulator for retaining drug stability and solubility, such as carbonic acid, acetic acid, oxalic acid, citric acid, phosphoric acid, hydrochloric acid, sodium hydroxide, arginine, lysine or salt thereof. In

addition, albumin, gelatin, citric acid, sodium ethylenediaminetetraacetate, dextrin, sodium hydrogen sulfite, polyol compounds such as polyethylene glycol, etc., as drug stabilizers, and p-oxybenzoates (e.g., methyl paraben, propyl paraben, etc.), benzyl alcohol, chlorobutanol, thimerosal etc., as preservatives, may be added.

The internal aqueous phase thus obtained is added to a solution containing a biodegradable polymer having a free carboxyl group at one end (hereinafter also referred to as polymer) (oil phase), followed by emulsification, to yield a W/O emulsion. This emulsification is achieved by a known dispersing method, such as the intermittent shaking method, the method using a mixer, such as a propeller stirrer or a turbine stirrer, the colloidal mill method, the homogenizer method or the ultrasonication method.

The above-described polymer-containing solution (oil phase) is prepared by dissolving a polymer in a substantially water-immiscible organic solvent. The water solubility of the organic solvent is preferably not higher than 3% (w/w) at normal temperature (20°C). Also, the boiling point of the organic solvent is preferably not higher than 120°C. Useful organic solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, trichloroethane, carbon tetrachloride, etc.), alkyl ethers having 3 or more carbon atoms (e.g., isopropyl ether, etc.), alkyl ester (4 or more carbon atoms) of fatty acids (e.g., butyl acetate, etc.), aromatic hydrocarbons (e.g., benzene, toluene, xylene, etc.) and the like. These solvents may be used in combination. The organic solvent is more preferably a halogenated hydrocarbon (e.g., dichloromethane, chloroform, chloroethane, trichloroethane, carbon tetrachloride, etc.), and still more preferably dichloromethane.

The polymer concentration in the oil phase varies, depending on the molecular weight of the polymer and the kind of solvent, and is preferably about 0.01 to about 80% (w/w), more preferably about 0.1 to about 70% (w/w), and still more preferably about 1 to about 60% (w/w).

In a sustained-release preparation, the content ratio of drug varies depending on the kind of drug, desired pharmacologic effect, duration of action and other factors, and is about 0.01 to about 50% (w/w), relative to the base biodegradable polymer. The ratio is preferably about 0.1 to about 40% (w/w), more preferably about 1 to about 30% (w/w).

Next, the W/O emulsion thus produced is subjected to in-water drying. The in-water drying method is carried out by adding the W/O emulsion to an aqueous phase (external aqueous phase) to yield a W/O/W emulsion, and removing the solvent from the oil phase.

The volume of the external aqueous phase is normally selected within the range from about 1 to about 10,000 times, preferably about 2 to about 5,000 times, and more preferably about 5 to about 2,000 times, that of the oil phase.

An emulsifier may be added to the external aqueous phase. The emulsifier may be any one, as long as it is capable of forming a stable W/O/W emulsion. Such emulsifiers include anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate, etc.), nonionic surfactants [e.g., polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals), etc.], polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin, hyaluronic acid and the like. Among these, a preferred emulsifier is polyvinyl alcohol. These emulsifiers may be used singly or in combination. Their

concentration can be chosen as appropriate over the range from about 0.001 to about 20% (w/w), preferably from about 0.01 to about 10% (w/w), and more preferably from about 0.05 to about 5% (w/w).

An osmotic pressure adjustor may also be added to the above external aqueous phase.

Any osmotic pressure adjustor can be used in the invention, so long as it produces osmotic pressure in an aqueous solution therof.

Examples of the osmotic pressure adjustor include water-soluble polyhydric alcohols; water-soluble monohydric alcohols; water-soluble monosaccharides, disaccharides and oligosaccharides or their derivatives; water-soluble amino acids; water-soluble peptides, proteins or their derivatives and the like.

Examples of the above water-soluble polyhydric alcohols include dihydric alcohols (e.g., glycerin, etc.), pentahydric alcohols (e.g., arabitol, xylitol, adonitol, etc.), hexahydric alcohols (e.g., mannitol, sorbitol, dulcitol, etc.) and the like. Among them, hexahydric alcohols, especially, mannitol is preferred.

Examples of the above water-soluble monohydric alcohols include methanol, ethanol, isopropyl alcohol and the like. Among them, ethanol is preferred.

Examples of the above water-soluble monosaccharides include pentoses (e.g., arabinose, xylose, ribose, 2-deoxyri-bose, etc.), hexoses (e.g., glucose, fructose, galactose, mannose, sorbose, rhamnose, fucose, etc) and the like. Among them, hexoses are preferred.

Examples of the above water-soluble disaccharides include maltose, cellobiose, α,α-trehalose, lactose, sucrose and the like. Among them, lactose and sucrose are preferred.

Examples of the above water-soluble oligosaccharides include trisaccharides (e.g., maltotriose, raffinose,

etc.), tetrasaccharides (e.g., stachyose, etc.) and the like. Among them trisaccharides are preferred.

Examples of the derivatives of the above monosaccharides, disaccharides and oligosaccharides include glucosamine, galactosamine, glucuronic acid, galacturonic acid and the like.

Examples of the above water-soluble amino acids include neutral amino acids such as glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, proline, hydroxyproline, cysteine, methionine and the like; acidic amino acids such as aspartic acid, glutamic acid and the like; basic amino acids such as lysine, arginine, histidine and the like. There can also be used salts of these water-soluble amino acids with acids (e.g., hydrochloric acid, sulfuric acid, phosphoric acid, etc.) or alkalis (e.g., alkaline metals such as sodium, potassium and the like, etc.).

Examples of the water-soluble peptides, proteins or their derivatives include casein, globulin, prolamine, albumin, gelatin and the like.

Among these materials, water-soluble polyhydric alcohols; and water-soluble monosaccharides, disaccharides and oligosaccharides or their derivatives are preferred, water-soluble polyhydric alcohols and water-soluble monosaccharides being more preferred and water-soluble polyhydric alcohols being most preferred.

These osmotic pressure adjustors can be used alone or in combination thereof. A concentration of the osmotic pressure adjustor is selected so that the tonicity of the external aqueous phase is about 1/50 to about 5 times, preferably about 1/25 to about 3 times, that of physiological saline. For example, when the osmotic pressure adjustors are non-inonic materials, the concentration of these osmotic pressure adjustors in the external aqueous phase is about 0.001% to about 60% (w/w), preferably about 0.01 to about 40% (w/w), more preferably

about 0.05 to about 30% (w/w), particularly preferably about 1 to about 10% (w/w). When the osmotic pressure adjustors are ionic materials, they are used in a concentration calculated by dividing the above concentration by the total ionic valency. The osmotic pressure adjustors may be added so that their concentration becomes more than their solubility, and a part of them may be dispersed.

In the production method of the present invention, it is preferable that during formation of a W/O/W emulsion, the viscosity of the W/O emulsion be adjusted to about 150 cp to about 10,000 cp. Viscosity-adjusting methods include (1) adjusting the biodegradable polymer concentration of the oil phase, (2) adjusting the volume ratio of aqueous and oil phases, (3) adjusting the temperature of the W/O emulsion (4) adjusting external aqueous phase temperature, (5) and adjusting the temperature of the W/O emulsion using a line heater, cooler, or the like, during injection of the W/O emulsion to the external aqueous phase. These methods may be used singly or in combination.

In essence, in the present method it is necessary to adjust the viscosity of the W/O emulsion to about 150 cp to about 10,000 cp when the W/O emulsion turns to a W/O/W emulsion.

With respect to (1) above, the biodegradable polymer concentration in the oil phase cannot definitely be determined, because it varies depending on the kind of biodegradable polymer, kind of organic solvent and other factors, but is preferably about 10 to about 80% (w/w).

With respect to (2) above, the volume ratio of the aqueous and oil phases also cannot definitely be determined, because it varies depending on kind and amount of drug and oil phase nature, but the W/O ratio is preferably about 1 to about 50% (v/v).

With respect to (3) above, the temperature of the W/O emulsion, if adjusted, falls within the range from about -20°C to the organic solvent's boiling point, preferably about 0 to about 30°C, and more preferably about 10 to about 20°C.

The viscosity of the W/O emulsion viscosity can be adjusted during production of the W/O emulsion, in cases (1) and (2) above.

With respect to (4) above, it is recommended that the temperature of the external aqueous phase be previously adjusted before the W/O emulsion is added thereto, to yield results similar to those obtained in (3) above.

The temperature of the external aqueous phase is about 5 to about 30°C, preferably about 10 to about 25°C, and more preferably about 12 to about 20°C.

Organic solvent can be removed by known methods, including the method in which the solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the solvent is evaporated while the degree of vacuum is adjusted using a rotary evaporator or the like.

The thus-obtained sustained-release preparation, in the form of e.g., microcapsules ("microcapsules" may be also referred to as "microspheres"), is centrifuged or filtered to separate its particles, which are then washed with distilled water several times to remove the free drug, drug support, emulsifier etc. adhering to the microcapsules surface, and again dispersed in distilled water etc. and lyophilized.

An anticoagulant may be added to the above lyophilization. The anticoagulant is exemplified by water-soluble polysaccharides such as mannitol and starches (e.g., corn starch), inorganic salts, amino acids, and

proteins. The anticoagulant is preferably mannitol. The mixing ratio (weight ratio) of the microcapsules and anticoagulant is about 50:1 to about 1:1, preferably about 20:1 to about 1:1, still more preferably about 10:1 to about 5:1.

To prevent mutual aggregation of particles during washing, an anticoagulant may be added to the distilled water for washing. The anticoagulant is exemplified by water-soluble polysaccharides such as mannitol, lactose, glucose and starches (e.g., corn starch, etc.), proteins such as glycine, fibrin, collagen, etc., and inorganic salts such as sodium chloride, sodium hydrogen phosphate, etc. The preferred anticoagulant is mannitol.

After lyophilization, the microcapsules may be heated under reduced pressure to further remove the water and organic solvent therefrom, where desired.

If the heating temperature is below the glass transition temperature of the biodegradable polymer component, the effect of inhibiting the initial burst of the bioactive peptide will not be obtained. Conversely, if the temperature is too high, the risk of aggregation and deformation of microcapsules and decomposition or degradation of the bioactive peptide will be increased. The heating time cannot be specified in general terms but can be determined in consideration of the physical properties (e.g. molecular weight, stability, etc.) of the component biodegradable polymer, species of bioactive peptide, particle average diameter of microcapsules, heating time, degree of desiccation of microcapsules and heating procedure.

As a preferred procedure, the microcapsules are heated at a temperature not below the glass transition temperature of the biodegradable polymer component and not so high as to cause aggregation of the microcapsules. For still better results, the heating temperature is preferably selected within the range from the glass transition

temperature of the biodegradable polymer component to about 30°C higher than the glass transition temperature of the component biodegradable polymer. Here, glass transition temperature is defined as the intermediate glass transition temperature determined using a differential scanning calorimeter during heating at a rate of 10 or 20°C per minute.

The heating time is also dependent on the heating temperature and the batch size of microcapsules, among other factors. Generally speaking, however, the heating time is preferably about 24 to about 120 hours, still more preferably about 48 to about 120 hours, after the microcapsules themselves have reached the specified temperature.

The heating method is not critical but any procedure conducive to a uniform heating of microcapsules can be employed.

As specific examples of such procedure, there may be mentioned heating in a constant-temperature bath, a fluidized bed, a moving bed or a kiln, and microwave heating. The most preferred method is heating in a constant-temperature bath.

By heating the microcapsules under reduced pressure after lyophilization, as stated above, the organic solvent is efficiently removed from the microcapsules, resulting in a biologically safe microcapsules. The residual organic solvent in the thus-obtained microcapsules is not more than about 100 ppm.

The thus-obtained microcapsules can be administered, as such or in the form of various dosage forms of non-oral preparations (e.g., intramuscular, subcutaneous or visceral injections or indwellable preparations, nasal, rectal or uterine transmucosal preparations, etc.) or oral preparations (e.g., capsules such as hard capsules and soft capsules, etc.), or solid preparations such as granules and

powders, or liquid preparations such as syrups, emulsions and suspensions.

An injectable preparation can be prepared by, for example, suspending microcapsules in water, along with a dispersing agent (e.g., Tween 80, HCO-60, carboxymethyl cellulose (including carboxymethyl cellulose sodium), sodium alginate, etc.), a preservative (e.g., methyl paraben, propyl paraben, etc.), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose, etc.) etc., to yield an aqueous suspension, or by dispersing it in a vegetable oil such as sesame oil or corn oil, or the like, to yield an oily suspension, whereby a practically usable sustained-release preparation is obtained.

When microcapsules are used as an injectable suspension, for instance, their average particle size is chosen over the range from about 0.1 to about 500 μm, as long as the requirements concerning degree of dispersion and needle passage are met. Preferably, the average particle size is about 1 to about 300 μm, and more preferably about 2 to about 200 μm.

When the sustained-release preparation is microcapsules, by adding the osmotic pressure adjustor as mentioned above, its particle shape become better spheric shape which is better for needle pasage.

Methods of preparing microcapsules as a sterile preparation include, but are not limited to, the method in which the entire production process is sterile, the method in which gamma rays are used as sterilant, and the method in which an antiseptic is added.

The sustained-release preparation of the present invention is not significantly toxic and can be used safely in mammals (e.g., humans, bovines, swines, dogs, cats, mice, rats, rabbits, etc.).

Although varying widely depending on kind, content and dosage form, and duration of release of the drug, target

disease (e.g., hormone-dependent diseases such as prostatic cancer, prostatic hypertrophy, endometriosis, uterine myoma, precocious puberty, breast cancer, bladder cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, gastritis, hodgkin's disease, malignant melanoma, metastasis, multiple myeloma, non-hodgkin's leukemia, non-small-cell lung cancer, ovarian cancer, peptic ulcer, serious fungal infection, small-cell lung cancer, valvular heart disease, mastopathy, polycystic ovary, infertility, controlled induction of ovaulation in women with chronic anovulation, acne, amenorrhea (e.g., secondary amenorrhea), ovarian and mammary cystic disease (including polycystic ovarian diseasea), gynecological cancer, ovarian hyperandrogenism and hirsutism, AIDS by rejuvenating the thymus to produce T-cells, male contraceptives for treatment of male sex offenders and in contraception, symptomatic relief of the premenstrural syndrome (PMS), in vitro fertilization), subject animal species and other factors, the dose of the sustained-release preparation may be set at any level, as long as the desired effect of the drug is obtained. The dose of the drug per administration can be chosen as appropriate over the range from about 0.01 mg to about 100 mg/kg body weight, preferably from about 0.05 mg to about 50 mg/kg body weight, and more preferably from about 0.1 mg to about 10 mg/kg body weight per adult, in the case of a 1-month release preparation.

The dose of the sustained-release preparation per administration can be chosen as appropriate within the range from about 0.1 mg to about 500 mg/kg body weight, preferably from about 0.2 mg to about 300 mg/kg body weight per adult. The frequency of administration can be chosen as appropriate, depending on kind, content and dosage form, duration of release of the drug, target disease, subject animal species and other factors, e.g., once every several weeks, once every month or once every several months.

Best Mode for Carrying out the Invention

The present invention is hereinafter described in more detail by means of the following reference examples and working examples, which are not to be construed as limitative. In the examples below, % values are by weight, unless otherwise stated.

Example 1

500 mg of the acetate (produced by TAP Company) of N-(S)-2-tetrahydrofuroyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH$_2$ (hereinafter referred to as peptide A) was dissolved in 0.6 ml of distilled water. The resulting solution was added to a solution of 4.5 g of a lactic acid-glycolic acid copolymer (hereinafter referred to as PLGA) [produced by Wako Pure Chemical, Japan, lot. 940810; lactic acid/glycolic acid (molar ratio), 74/26, weight-average molecular weight based on GPC, 10,000; number-average molecular weight based on GPC, 3,900; number-average molecular weight based on terminal group quantitation, 3,700] in 5.8 ml of dichloromethane, followed by homogenization for 60 seconds in a small homogenizer (produced by Kinematica Company) to yield a W/O emulsion. After being cooled to 16°C, the W/O emulsion was poured over 1,000 ml of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 16°C, and then prepared as a W/O/W emulsion using a turbine type homomixer (produced by Tokushu Kika) at 7,000 rpm. This W/O/W emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the W/O emulsion, which was then centrifuged at 2,000 rpm using a centrifuge (05PR-22, Hitachi Limited). The resulting precipitate was again dispersed in distilled water, followed by centrifugation and washing down of the free drug. After the collected microcapsules were again

dispersed in a small amount of distilled water, 0.3 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 9.5% (w/w), respectively.

Example 2

Microcapsules were obtained in the same manner as in Example 1, except that PLGA [produced by Wako Pure Chemical, lot. 940813; lactic acid/glycolic acid (molar ratio), 73/27; weight-average molecular weight based on GPC, 13,000; number-average molecular weight based on GPC, 4,500; number-average molecular weight based on terminal group quantitation, 4,700] was used. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 9.5% (w/w), respectively.

Example 3

Microcapsules were obtained in the same manner as in Example 1, except that PLGA [produced by Wako Pure Chemical, lot. 940808; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 7,800; number-average molecular weight based on GPC, 3,500; number-average molecular weight based on terminal group quantitation, 3,000) was used. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 9.5% (w/w), respectively.

Example 4

Microcapsules were obtained in the same manner as in Example 1, except that the amount of peptide A acetate was 794 mg. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 14.3% (w/w), respectively.

Example 5

15 g of peptide A acetate was dissolved in 18 ml of distilled water. The resulting solution was added to a solution of 135 g of PLGA [produced by Wako Pure Chemical, lot. 940810; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 10,000; number-average molecular weight based on GPC, 3,900; number-average molecular weight based on terminal group quantitation, 3,700) in 174 ml of dichloromethane, followed by homogenization in a homogenizer to yield a W/O emulsion. This W/O emulsion was poured over 30 l of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 17°C, and was then prepared as a W/O/W emulsion using an in-line type homomixer. This W/O/W emulsion was stirred at room temperature to volatilize off the dichloromethane and solidify the W/O emulsion, which was then centrifuged. The resulting precipitate was washed with distilled water to remove the free drug. After the collected microcapsules were again dispersed in a small amount of distilled water, 13.5 g of D-mannitol was added to the dispersion, which was lyophilized and then dried under reduced pressure in a constant-temperature chamber at 40-43°C for 19 hours, then at 42-44°C for 48 hours to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 3-60 μm and 8.7% (w/w), respectively.

Example 6

Microcapsules were obtained in the same manner as in Example 1, except that the acetate of NAcD2Nal-D4ClPhe-D3Pal-Ser-Tyr-DhArg(Et_2)-Leu-hArg(Et_2)-Pro-DAlaNH$_2$ (produced by Syntex Company) was used in place of peptide A acetate. The particle size distribution and peptide content of the microcapsules were 5-60 μm and 9.4% (w/w), respectively.

Example 7

857 mg of peptide A acetate was dissolved in 0.8 ml of distilled water. The resulting solution was added to a solution of 4.5 g of PLGA [produced by Wako Pure Chemical, lot. 950526; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 11,700; number-average molecular weight based on GPC, 5,200; number-average molecular weight based on terminal group quantitation, 3,800) in 6 ml of dichloromethane, followed by homogenization in a homogenizer to yield a W/O emulsion. Microcapsules were obtained in the same manner as in Example 1, except that 0.5 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 11.7% (w/w), respectively

Example 8

Microcapsules were obtained in the same manner as in Example 1, except that the amount of peptide A acetate was 1125 mg, the amount of distilled water was 1.0 ml, the amount of dichloromethane was 6.3 ml. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 11.7% (w/w), respectively.

Example 9

Microcapsules were obtained in the same manner as in Example 7, except that the amount of peptide A acetate was 1421 mg, the amount of distilled water was 1.2 ml, the amount of dichloromethane was 6.7 ml. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 17.5% (w/w), respectively.

Example 10

Microcapsules were obtained in the same manner as in Example 8, except that 50 g of D-mannitol was added to

1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 14.7% (w/w), respectively.

Example 11

Microcapsules were obtained in the same manner as in Example 9, except that 50 g of D-mannitol was added to 1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 17.0% (w/w), respectively.

Reference Example 1

1125 mg of peptide A and 4.5 g of PLGA [produced by Wako Pure Chemical, lot. 950526; lactic acid/glycolic acid (molar ratio), 74/26, weight-average molecular weight based on GPC, 11,700; number-average molecular weight based on GPC, 5,200; number-average molecular weight based on terminal group quantitation, 3,800] were dissolved in 6.0 ml of dichloromethane. After being cooled to 16°C, the solution was poured over 1,000 ml of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 16°C, and then prepared as a O/W emulsion using a turbine type homomixer (produced by Tokushu Kika) at 7,000 rpm. This O/W emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane, which was then centrifuged at 2,000 rpm using a centrifuge (05PR-22, Hitachi Limited). The resulting precipitate was again dispersed in distilled water, followed by centrifugation and washing down of the free drug. After the collected microcapsules were again dispersed in a small amount of distilled water, 0.5 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 13.2% (w/w), respectively.

Reference Example 2

Microcapsules were obtained in the same manner as in Reference Example 1, except that the amount of peptide A acetate was 1421 mg, the amount of dichloromethane was 6.2 ml. The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 15.9% (w/w), respectively.

Reference Example 3

Microcapsules were obtained in the same manner as in Reference Example 2, except that 50 g of D-mannitol was added to 1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol The particle size distribution and peptide A content of the microcapsules were 5-60 μm and 15.5% (w/w), respectively.

Experimental Example 1

About 20 mg of the microcapsules obtained in Example 4 was dispersed in 0.5 ml of dispersing solvent (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol dissolved therein), and injected subcutaneously to the backs of male SD rats at 10 weeks of age, using a 22-G injection needle. After administration, rats were sacrificed at constant intervals; the remaining microcapsules were taken out from the injection site; microcapsules peptide A quantitation results are shown in Table 1.

Table 1

Time after Administration	Ratio of Residual Peptide A (%)
1 day	96.4
1 week	84.8
2 weeks	59.2
3 weeks	38.8
4 weeks	24.6

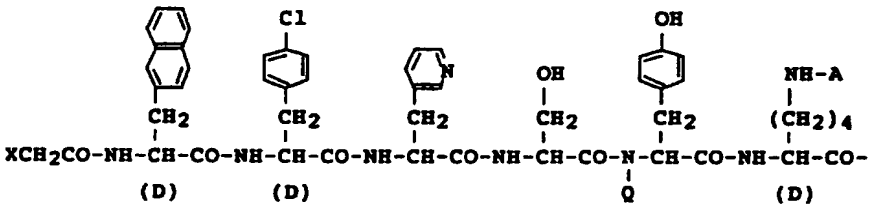
As shown in Table 1, the microcapsules obtained according to the production method of the present invention release peptide A constantly, with substantially no initial burst.

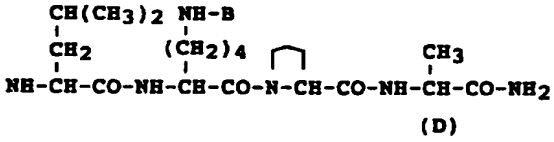
Industrial Applicability

According to the present Invention, a sustained-release preparation containing peptide [I] or a salt thereof can be obtained easily and at high recover rates.

CLAIMS

1. A method of producing a sustained-release preparation, which comprises producing a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by the formula:





wherein X represents a hydrogen atom or a tetrahydrofuryl-carboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion.

2. A method of claim 1, wherein the biodegradable polymer is an aliphatic polyester.

3. A method of claim 2, wherein the aliphatic polyester is a lactic acid-glycolic acid copolymer.

4. A method of claim 3, wherein a composition ratio of lactic acid and glycolic acid is about 100/0 to about 40/60 (mole%).

5. A method of claim 3, wherein a weight-average molecular weight of the copolymer is about 5,000 to about 25,000.

6. A method of claim 1, wherein a peptide concentration in the internal aqueous phase is about 0.1 to about 150% (w/v).

7. A method of claim 1, wherein a polymer concentration in the oil phase is about 0.01 to about 80% (w/w).

8. A method of claim 1, wherein a volume ratio of the internal aqueous and oil phase is about 1 to about 50% (v/v).

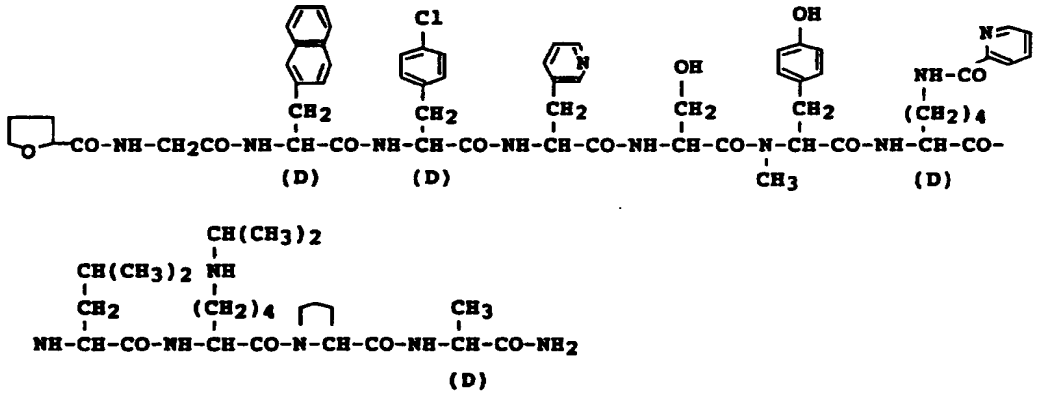
9. A method of claim 1, wherein a volume of the external aqueous phase is about 1 to about 10,000 times that of the oil phase.

10. A method of claim 1, wherein the preparation is microcapsules.

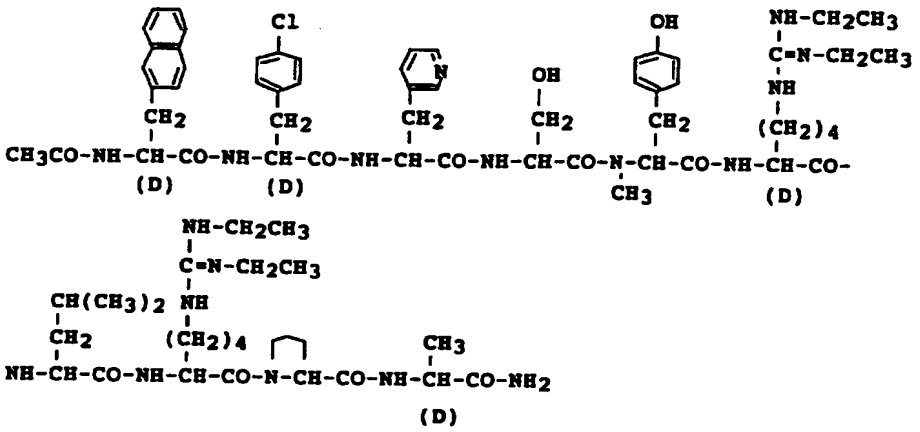
11. A method of claim 1, wherein X is 2-tetrahydrofurylcarboxamido.

12. A method of claim 11, wherein the 2-tetrahydrofurylcarboxamido is (2S)-tetrahydrofurylcarboxamido.

13. A method of claim 1, wherein the peptide is of the formula:



14. A method of claim 1, wherein the peptide is of the formula:



15. A sustained-release preparation, which is produced by the method of claim 1.

16. A preparation of claim 15, wherein a content ratio of the peptide is about 0.01 to about 50% (w/w), relative to the polymer.

17. A preparation of claim 15, wherein the preparation is microcapsules.

18. A preparation of claim 17, wherein the microcapsules are for injection.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 96/00090

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/09 A61K9/16 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 601 799 (TAKEDA CHEMICAL INDUSTRIES LTD.,JP) 15 June 1994 cited in the application see claims see page 11, line 17 - line 46	1-18
A	EP,A,0 413 209 (ABBOTT LABORATORIES,U.S.A.) 20 February 1991 cited in the application see claims see page 20, line 14 - line 49	1-18

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 May 1996	15.05.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Scarponi, U

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inv[illegible]tional Application No
PCT/JP 96/00090

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-601799	15-06-94	AU-B-	5211393	16-06-94
		CA-A-	2110730	08-06-94
		FI-A-	935471	08-06-94
		WO-A-	9413317	23-06-94
		JP-A-	7097334	11-04-95
		NO-A-	934423	08-06-94
		NZ-A-	250349	22-12-94
		US-A-	5480868	02-01-96
		CN-A-	1096221	14-12-94
		LT-A,B	1526	25-10-94
		ZA-A-	9309163	07-06-95
EP-A-413209	20-02-91	US-A-	5110904	05-05-92
		AU-B-	5789294	26-05-94
		AU-B-	6028690	07-02-91
		CA-A-	2022444	08-02-91
		JP-A-	3101695	26-04-91
		US-A-	5300492	05-04-94